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Manuscripts and editorial correspondence should be addressed to

Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
H-2462 Martonvásár, Hungary
Phone: (36-22) 569-521
Fax: (36-22) 460-213
E-mail: actaagr@mail.mgki.hu

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TUDOMÁNYOS AKADÉMIA
KÖNYVTÁRA

*Paper presented at the scientific meeting entitled "Role of Crop Production in the Multifunctional Agriculture of the Future", held to celebrate the 50th anniversary of Acta Agronomica Hungarica in Martonvásár, Hungary on 19th November 2002.

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EFFECT OF SALINITY ON THE PHYSIOLOGICAL RESPONSES OF SELECTED LINES/VARIETY OF WHEAT

A. M. ISMAIL

BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH OF VALLEY UNIVERSITY, QENA, EGYPT

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The effect of different concentrations of salinity (NaCl up to 250 mM) was studied on the germination, dry matter production and some relevant metabolic parameters of two lines (Sakha 69 and Sakha 164) and one variety (Stork) of wheat (*Triticum aestivum* L.). During the germination and seedling stages the experimental lines tolerated lower and moderate doses of salinity, while the variety was significantly retarded at the lower and moderate levels and completely inhibited at higher doses of salinity. The water content remained more or less unchanged in the two lines under saline conditions, whereas in Stork increasing salinity resulted in a significant decrease in water content. A stimulation of the net photosynthetic rate in both lines, Sakha 69 and Sakha 164, was observed at moderate salinity, but the highest levels proved to be inhibitory. In the Stork variety all salinization levels inhibited photosynthetic activity. The respiration rate in the two tested lines was influenced from salinity levels of 150 mM upwards and increased progressively with the salinity level. In Stork plants increasing salinity levels increased the respiration rate. The soluble sugar and soluble protein contents of the lines increased with increasing salinity. The opposite pattern was revealed in the case of Stork. The amino acid content, including proline, increased significantly with an increase in salinity in all tested plants. The potassium/sodium ratio decreased significantly with a rise in salinization.

Key words: germination, salinity, tolerance, proline, photosynthetic rate, respiration rate

Introduction

Higher plant species differ widely in their tolerance to salinity, which depends primarily on the morphological features of the plant, the uptake and transport of salt, and physiological and metabolic events at the cellular level (Winicov, 1993). Depending on the species, different strategies are employed to induce salt tolerance (Weimberg and Shannon, 1988; Kalaji and Nalborczyk, 1991). These differences in salt tolerance exist not only between different genera and species, but also between varieties. For example, there are reports on the specific responses of different wheat varieties to salinity (Francois et al., 1986).

The adverse effects of salt stress on the seed germination, seedling growth and some relevant metabolic processes of a number of cultivated plant species have been subjected to extensive investigations (Cheeseman, 1988; Chartzoulakis, 1992). The trends and magnitudes of these physiological activities varied according to the level and duration of the water stress induced by NaCl salinity treatments, as well as the plant type used. This variation in plant response and the need to select economic crops for cultivation in saline soils necessitated a series of investigations to test their ability to tolerate salinity and to adapt to possible changes in their physiological activities under salinization treatments. The differences in salt tolerance between plant species cannot be attributed to differences in specific ion effects, but may be related to

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factors that reduce the net assimilation rate of species during the early stages of growth (He and Cramer, 1993).

The present experiment was focused on the effect of various degrees of salinity on seed germination and seedling growth, and on the relevant metabolic processes of three wheat genotypes.

Materials and methods

The effect of osmotic stress induced by salinity on the seed germination and seedling growth of wheat (*Triticum aestivum* L.) lines Sakha 69, Sakha 164 and wheat variety Stork was studied in germination experiments performed as described by Maftoun and Sepaskhah (1978). The following osmotic stress levels were used: 0 (control), 50, 100, 150, 200 and 250 mM NaCl salinity. Fifteen seeds were placed on absorbent pads in Petri dishes to which 30 ml of the experimental solution was added. Three replicates (Petri dishes) were prepared for each treatment. Seeds were considered to be germinated after the radicle emerged from the testa. After two weeks of germination, the seedlings were dried in an aerated oven at 70°C to constant dry weight. The contents of chlorophylls a and b and carotenoids were determined spectrophotometrically (Metzner et al., 1965). Net photosynthesis (oxygen evolution) and dark respiration (oxygen consumption) were determined manometrically using the Warburg method (Umbreit et al., 1959). For the determination of soluble sugars, a known weight of powdered tissue was extracted with distilled water for two hours on a boiling water bath. After cooling, the extract was filtered and the filtrate was filled up to a definite volume, after which the water-soluble sugars were determined by the anthrone sulphuric acid method (Fales, 1951). Free amino acids were determined according to the method of Moore and Stein (1948), free proline according to the method of Bates et al. (1973) and soluble protein according to Lowry et al. (1951). Sodium and potassium were determined by the flame photometer method (Williams and Twine, 1960) and calcium and magnesium according to Schwarzenbach and Biedermann (1948).

The data of all experiments were subjected to analysis by the least significant difference test (L.S.D.).

Results

The data in Table 1 reveal that the final germination percentage of wheat lines Sakha 69 and Sakha 164 remained more or less constant up to the level of 200 mM NaCl, whereas in the variety Stork the final germination percentage decreased sharply with increasing salt stress and was completely inhibited at 200 and 250 mM NaCl. Concerning the dry matter production of seedlings of Sakha 69 and Sakha 164, it seems that salinity had no effect on the dry matter except at the highest level used (250 mM), where these values were significantly reduced. It is interesting to note that 50 and 100 mM NaCl resulted in an increase in dry matter in Sakha 69 and Sakha 164 as compared with that of the control (Table 1). On the other hand, increasing salinity caused a significant reduction in dry matter yield in Stork. The tissue water content of the two lines increased with increasing water stress up to 100 mM NaCl; it then remained more or less constant up to 200 mM NaCl, and thereafter the values decreased sharply as compared with those of control seedlings. In Stork the tissue water content decreased sharply with an increase in the salinity concentration in the culture medium. Moreover, Stork seedlings had a much lower content even in control seedlings than in the experimental lines.

Salinity induced a significant increase in the content of photosynthetic pigments up to 150 mM NaCl for both lines. Above this level, the values decreased with an increase in the NaCl concentration as compared with the control (Table 1). In the case of Stork, the photosynthetic pigment content was highly significantly reduced at all salinity levels used, as compared with that of plants grown without salinity (control). Salinity concentrations of 50 and 100 mM NaCl stimulated the net photosynthetic rate of both tested lines but at higher concentrations a significant decrease was recorded. In Stork all the salinization levels induced a significant decrease in photosynthetic activity (Table 1). The respiration rate of the two tested lines remained more or less comparable to that of the control up to 150 mM NaCl; above this level the respiration rates significantly increased with a rise in the salinity level. In the variety Stork, the respiration rate increased significantly with increasing salinity stress.

Table 1

Effects of different concentrations (mM) of NaCl on seed germination (SG, %), dry matter (DM, g plant^{-1}), water content (WC, %), contents of chlorophyll (a, b and carotenoids) (g kg^{-1} d.m.), net photosynthesis (P_N) and dark respiration (R_D) rates (μmol O_2 kg^{-1} d.m.) in seedlings of selected lines/variety of wheat plants (Sakha 69, 164 and Stork)

Treatments	SG	DM	WC	Chl a	Chl b	Car.	P_N	R_D
Sakha 69								
Control	100	1.02	71.0	5.77	3.80	3.55	340	70.4
50	100	1.34**	71.5	7.12**	4.20*	3.80*	350	72.5
100	100	1.29**	72.5*	7.88**	4.85**	3.43	361**	75.2
150	100	1.08	71.2	7.85*	4.41**	3.44	330	77.3*
200	95	0.74**	70.0	5.21	3.90	2.88**	305**	86.0**
250	85**	0.67**	66.4**	3.65**	3.22**	2.10**	244**	103.3**
L.S.D.$_{5\%}$	9.55	0.18	1.15	0.75	0.36	0.24	12.40	5.70
L.S.D.$_{1\%}$	12.90	0.24	1.55	1.01	0.49	0.32	16.50	7.60
Sakha 164								
Control	100	0.85	74.5	4.60	2.80	2.71	230	66.8
50	100	1.01*	75.5	5.10	3.50**	3.00*	265**	67.5
100	100	0.94	75.8	4.83	2.90	2.75	257**	71.5*
150	95	0.77	73.7	4.88	2.75	2.56	231	80.2**
200	95	0.54**	70.0**	3.77**	2.50**	1.88**	195**	93.7**
250	80**	0.49**	67.5**	3.10**	2.15**	1.08**	163**	107.2**
L.S.D.$_{5\%}$	10.0	0.13	2.17	0.55	0.21	0.23	14.80	4.22
L.S.D.$_{1\%}$	13.5	0.18	2.93	0.74	0.28	0.31	20.00	5.70
Stork								
Control	100	0.66	65.5	4.22	2.40	2.55	185	56.1
50	55**	0.59	60.2**	3.77	2.03	1.88**	146**	68.5**
100	40**	0.45**	51.0**	3.02**	1.75**	1.65**	130**	88.4**
150	40**	0.38**	33.5**	2.55**	1.58**	1.20**	101**	96.4**
200	IE	IE	IE	IE	IE	IE	IE	IE
250	IE	IE	IE	IE	IE	IE	IE	IE
L.S.D.$_{5\%}$	12.44	0.11	2.33	0.47	0.17	0.14	6.77	3.88
L.S.D.$_{1\%}$	16.80	0.15	3.15	0.63	0.23	0.19	9.14	5.24

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with the control. IE: Injurious effects; plants failed to survive.

It can be seen from the results in Table 2 that the contents of soluble sugars in the seedlings of lines 69 and 164 increased progressively as the salinity concentration increased up to the highest level used. In Stork seedlings the soluble sugars content decreased significantly with increasing water stress. The content of soluble proteins in the seedlings of wheat lines increased considerably with increasing salt stress in the culture medium. The opposite pattern was exhibited in the case of Stork. The free amino acid content, including free proline, also tended to increase in the tested plants with increasing salt stress in the culture medium.

The sodium content in variously salinized plants (lines 69, 164 and Stork variety) rose progressively with increasing salinity (Table 3). Salinity stress markedly raised the content of potassium in all tested plants, except in lines 69 and 164 grown at 250 mM NaCl and Stork variety seedlings grown at 150 mM NaCl, where these contents were lower in comparison with those of control plants. The calcium and magnesium contents in the lines did not increase up to 150 mM NaCl except for Mg^{2+} in Sakha 164. In Stork the Ca^{2+} and Mg^{2+} contents decreased progressively with increasing salinity levels.

Table 2

Effects of different concentrations (mM) of NaCl on the soluble sugar, soluble protein, free amino acid and proline contents (g kg^{-1} d.m.) in seedlings of selected lines/variety of wheat plants (Sakha 69, 164 and Stork)

Treatments	Soluble sugar	Soluble protein	Free amino acids	Proline
Sakha 69				
Control	20.25	10.55	2.50	1.88
50	26.20**	11.03	2.90	2.50**
100	31.20**	11.45	3.60**	3.00**
150	33.40**	15.09**	3.85**	3.15**
200	33.00**	14.11**	4.30**	3.70**
250	32.55**	15.04**	4.10**	3.45**
L.S.D.$_{5\%}$	2.77	2.05	0.61	0.49
L.S.D.$_{1\%}$	3.74	2.73	0.82	0.66
Sakha 164				
Control	23.20	9.77	4.39	2.55
50	23.50	9.89	5.90	3.00
100	24.20	11.50*	5.15*	3.66**
150	26.40**	11.39*	6.66**	4.10**
200	25.40*	12.07**	5.40**	4.30**
250	25.55*	11.50*	5.30**	4.18**
L.S.D.$_{5\%}$	1.85	1.55	0.71	0.54
L.S.D.$_{1\%}$	2.50	2.06	0.96	0.73
Stork				
Control	17.70	5.80	2.10	1.86
50	14.20**	5.10**	3.95**	2.68**
100	11.10**	3.60**	3.40**	2.00**
150	9.40**	3.25**	3.25**	2.30**
200	IE	IE	IE	IE
250	IE	IE	IE	IE
L.S.D.$_{5\%}$	2.03	0.44	0.36	0.25
L.S.D.$_{1\%}$	2.70	0.60	0.49	0.34

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with the control. IE Injurious effects; plants failed to survive.

Table 3
Effects of different concentrations (mM) of NaCl on the contents of Na, K, Ca and Mg ($g\ kg^{-1}$ d.m.) and the K/Na ratio in seedlings of selected lines/variety of wheat plants (Sakha 69, 164 and Stork)

Treatments	Na	K	K/Na	Ca	Mg
Sakha 69					
Control	10.25	16.07	1.57	7.77	3.44
50	11.36	17.50	1.54	9.70**	4.10*
100	18.08**	19.75**	1.09**	8.71*	3.70
150	27.18**	22.73**	0.84**	6.88*	2.91*
200	34.05**	20.45**	0.60**	6.55**	2.52**
250	39.70**	14.88**	0.37**	4.22**	2.13**
$L.S.D._{5\%}$	3.40	2.05	0.16	0.75	0.50
$L.S.D._{1\%}$	4.56	2.75	0.22	1.00	0.68
Sakha 164					
Control	9.74	12.66	1.30	7.40	2.10
50	12.96*	14.30	1.10*	7.85	3.55**
100	23.55**	16.01**	0.68**	6.88**	2.60**
150	33.20**	14.30**	0.43**	4.70**	2.55**
200	37.90**	14.77**	0.39**	4.55**	1.70**
250	36.80**	11.10**	0.30**	2.65**	1.15**
$L.S.D._{5\%}$	2.90	1.80	0.19	0.60	0.23
$L.S.D._{1\%}$	3.92	2.43	0.26	0.81	0.31
Stork					
Control	10.03	11.34	1.13	5.20	2.75
50	19.55**	11.70	0.60**	4.22**	2.20**
100	24.88**	12.12	0.49**	2.88**	1.15**
150	28.85**	10.18*	0.35**	1.30**	0.96**
200	IE	IE	IE	IE	IE
250	IE	IE	IE	IE	IE
$L.S.D._{5\%}$	2.05	1.08	0.14	0.40	0.21
$L.S.D._{1\%}$	2.77	1.55	0.19	0.54	0.28

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with the control. IE: Injurious effects; plants failed to survive.

Discussion

The results obtained in the present work revealed that during germination wheat line Sakha 69 can tolerate salinity up to the level of 150 mM NaCl and Sakha 164 up to the level of 100 mM. However, in the case of the variety Stork germination exhibited greater sensitivity and the seedlings failed to survive under moderate and high levels of salinity. Tolerance up to these levels was closely associated with a relatively stable water content and dry matter yield in the seedlings of the three wheat genotypes. In this respect, there was a significant decrease in these values with rising salinity levels as compared with the control (0 mM NaCl). This inhibitory effect of salinity on seed germination and seedling growth may be attributed to the accumulation of toxic ions and/or reduced water uptake, which arrests radicle emergence (Bengum et al., 1992; Lopez et al., 1994; Lin and Kao, 1996).

The present results showed that dark respiration remained more or less unchanged in the two experimental lines, whereas at levels which caused a reduction in dry matter yield, the rate of respiration considerably increased. Thus, the increase in respiration seems to be linked to the salt stress (Pandey and Divate, 1976; Zagdanska, 1984). In Stork, salinity induced a progressive increase in the rate of dark respiration. This enhancement in the respiration of salinized plants could be due to the need for higher energy allocation for the maintenance of osmotic adjustment, ion concentration gradient and active transport processes for the repair of tissues (Loeblich, 1982; Hamada, 1990).

The adverse effects of salinity stress on the photosynthetic pigments and photosynthetic activity of the tested lines and variety were most pronounced at the 200 mM NaCl level in the case of line 69 and at the 100 mM NaCl level in the case of line 164, whereas in the case of Stork these values exhibited a progressive decrease at all salinity levels. This inhibitory effect is in agreement with that reported by Kalaji and Nalborczyk (1991). The reduction in pigment biosynthesis due to salt stress could be attributed to structural changes in the photosynthetic apparatus (Sharma and Hall, 1991; Hernandez et al., 1995). In this connection, Meinzer et al. (1994) reported that photosynthesis was impaired by the biochemical capacity (mesophyll conductance) rather than by stomatal conductance at high salinity levels. Although reduced leaf photosynthesis could fully explain the observed growth reduction at high salinity, the minor effects of other factors such as increased respiration (Taleisnik, 1987) cannot be excluded.

In wheat lines 69 and 164, salinity induced a progressive increase in the amounts of soluble sugars as well as in protein. In the case of Stork, the opposite effect occurred. Drossopoulos et al. (1987) and Kameli and Losel (1995) reported that an increase in these soluble components may in turn play an important role in increasing the osmotic pressure of the cytoplasm and in the alleviation of the imposed salt stress. Soluble sugars are considered as the principle osmotica in many glycophytes subjected to saline conditions (Greenway and Munns, 1980). Salinity stress was found to induce an accumulation of soluble sugars in crop plants (Schubert et al., 1995).

Lines 69 and 164 (more salt-tolerant) accumulated more proline compared to the variety Stork (less salt-tolerant). The rapid accumulation of proline in plant tissue is one of the most notable metabolic consequences of salinity stress (Lin and Kao, 1996; Morabito et al., 1996). The pattern of changes in amino acids was opposite to that of proline, indicating that the increase in proline occurs at the expense of other amino acids through the effect of salinity in promoting their conversion (Boggess et al., 1976). Therefore, it may be concluded that the accumulation of proline and other free amino acids offers great promise as one of the major physiological mechanisms of salt tolerance in both tested lines.

The sodium content increased with increasing salinity in the culture medium. The other detected minerals (K^+, Ca^{2+} and Mg^{2+}) remained more or less unchanged irrespective of the salinity level and the genotype. However, the extent of sodium accumulation varied among the three genotypes of wheat. Plants differ in their strategy towards Na accumulation under salinity. The experimental genotypes contained appreciable amounts of K^+, Mg^{2+} and Ca^{2+} at moderate levels of salinity, indicating their partial resistance to low salt stress. The contents of Ca^{2+} and Mg^{2+} were low, especially in Stork, as compared with the high contents of Na^+ and K^+. Reed et al. (1981) suggested that the distribution of both divalent cations is passive, or requires an active efflux to produce the low values observed for intercellular concentrations. This suggests that the osmoregulatory role of both cations was to increase the tissue water content, which in turn might play a role in the Na efflux of these plants. Also, the increase in potassium content in lines 69 and 164 might play a role in increasing the salt tolerance of both lines compared with Stork, which is a sensitive cultivar. Janardan et al. (1976) and Ismail (1996) found that the content of K^+ was higher in salt-tolerant than in salt-sensitive cultivars and recommended it as a suitable selection criterion for salt tolerance. In this respect, Salama et al. (1994) concluded that salt tolerance is accompanied by the maintenance of K^+ content in wheat, which may result in the effective protection of the chloroplast structure. In addition, Erdei et al. (1996) reported that sorghum (more tolerant to stress) possessed the capability for maintaining higher K^+ levels than maize (less tolerant to stress) under salt stress.

In the light of the experimental results and the parameters studied in this work, it can be concluded that the tolerance of the two wheat lines 69 and 164 might be linked to the stimulus of net photosynthesis and the accumulation of soluble carbohydrates, and to increases in soluble proteins, proline content and the potassium/sodium ratio, leading to an improvement in their ability to absorb water at moderate salinity levels. Plants of the variety Stork exhibited greater sensitivity to salt stress.

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CONTRIBUTION OF POPULATION IMPROVEMENT TO THE DEVELOPMENT OF MAIZE LINES WITH COMMERCIAL VALUE

G. HADI

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The grain yield was increased by 8.2% per cycle (32.8% overall) in a population of Mindszentpusztai Yellow Dent (MYD), by 8.9% per cycle (35.6% overall) in a population of Mv Syn. I and by 4.9% per cycle (19.7% overall) in a population of Westigua when tested on the closed pedigree line HMv 124-2. Averaged over the three populations the rise in grain yield was 7.5% per cycle, giving a total of around 30% after four cycles. The grain moisture at harvest showed a slight but significant decrease, while there was no change in the percentage stalk lodging. It seems probable that this increase in grain yield was achieved not at the expense of other correlated characters, but as the result of a greater frequency of gene combinations having a positive effect on grain yield, since recurrent selection was combined with selection for multiple ears at high plant density. The hybrid performance of the improved populations was extremely good, reaching 87.9% of that of commercial hybrids.

Over the last 25 years around 11,500 S_1 families have been tested from a total of 115 populations (including the three discussed above) and the inbreeding of the selected families was continued until the homozygous state was reached. Despite careful selection, it has not proved possible to breed inbred lines suitable for the development of hybrids with commercial value. Further research will be required to discover the reasons for this failure.

Key words: maize, population improvement, inbred line development

Introduction

According to Duvick (1977; 1992), Russell (1986) and other authors, the contribution of maize breeding to increases in yield averages is approx. 70 kg/ha/year. Many scientists expect this trend to increase, resulting in an improvement in the yield potential not only under good production conditions, but also when the crop is exposed to various abiotic and biotic stress conditions.

The introduction of hybrid maize breeding, according to the method of Shull (1908; 1909), had both advantages and disadvantages compared with variety breeding. While the agroeconomic, climatic and biotic conditions under which maize is produced undergo constant changes, the genetic background of cultivated hybrids is kept constant by variety maintenance. Hybrids adapted to new conditions and new challenges can only be bred if the parental components already possess the necessary gene combinations or if these can be produced by crossing.

The first lines with commercial value were developed in the 1920s and 1930s. According to Hallauer (1990) it was already obvious in the 1930s that the

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reselection of previously selected varieties would not result in lines better than those currently available. A solution was sought in two directions. Hayes and Johnson (1939) and Johnson and Hayes (1940) hoped to develop better lines by crossing elite lines with other elite lines using the pedigree method.

Jenkins (1935; 1940) and Sprague and Tatum (1942) suggested using elite lines to develop synthetic varieties which, together with other populations and gene pools, could then be improved by recurrent selection. In this way the chance of developing lines with better commercial value would increase from cycle to cycle. This method also differed from the former in that line components added to a population in the ratio of 1/12 or 1/16 would not erode the heterosis patterns to such a great extent.

This proved to be true in selection studies made in Iowa, where Russell (1991) obtained a yield increment of 72 kg/ha/year from the first ten cycles of reciprocal recurrent selection on populations of BSSS and BSCB1, a figure similar to that achieved with commercial hybrids. Duvick (1992) compared the gain achieved with the population pairs BS10 C_8/BS11 C_8 and PIAA C_5/PIBB C_5 with that of commercial hybrids grown during consecutive eras and recorded a yield increase of 117 kg/ha/year for the first population pair and 35 kg/ha/year for the second. It is promising that population improvement is capable of reducing the difference between the yields of initial sources and those of commercial hybrids.

Few data are available on the economically exploitable reserves remaining in various populations. Endre Pap, the breeder of the variety Mindszentpusztai Yellow Dent (MYD), began developing the variety in 1917 using the ear-to-row method. After the first 15 years he compared the results of the C_0, C_{10} and C_{15} cycles and found that there had been a yield increase of 20% over the first 10 cycles with a further 2% total increase over the following 5 cycles (cit.: Hegedüs, 1996). Gardner (1978) carried out a comparative selection study on the variety Hayes Golden. A maximum yield of around 140% of the C_0 level was obtained after both mass selection (control), irradiated mass selection, mass selection for multiple ears and mass selection using the ear-to-row method. When the methods were continued unchanged, yield declines were observed in later cycles. No doubt similar reasons were responsible for the results achieved with the variety Golden Glow by Maita and Coors (1996) and DeLeon and Coors (2002), who reached a yield plateau after 12 cycles when carrying out mass selection for multiple ears, while in later cycles not only was there no increase in yield, but there was a reduction in the weight of grains per ear and an increase in the number of sideshoots bearing ears. After 9 cycles of selection on Va 17 × Va 29 F_2, Genter (1972) also found that substantial yield increases could only be obtained in the first 3–4 cycles. Although the yield potential of lines originating from the C_8 selection cycle was promising, the author did not report on the development of lines with commercial value from this programme.

Russell (1983) compared lines B 14 and B 37 from the C_0 cycle of the BSSS population with line B 84 from the C_7 cycle using the tester Mo 17 and found that line B 84 gave a 27.3% higher yield than the lines from the C_0 cycle. No further data were found, however, in the compilation published by Gerdes et al. (1994) on any lines of commercial value arising after the C_7 cycle either from BSSS or in any other population improvement programme.

Selection studies generally report the results of 4–6 cycles and do not touch on the selection reserves, which are probably not infinite. In terms of grain yield they can probably be put at 120–160% of the C_0 level. Nevertheless, it appears that if populations with satisfactory performance and genetic variability are chosen or developed for recurrent selection, the size of the selection reserves does not limit the development of lines better than those currently available.

Investigations were made to determine what improvement could be achieved by means of recurrent selection in the grain yield and in other important agronomic traits of the three populations chosen on the basis of performance. A further aim was to determine whether lines which could be used to develop hybrids with commercial value were generated more frequently from improved cycles.

Materials and methods

The variety Mindszentpusztai Yellow Dent (MYD) was bred by Endre Pap using the ear-to-row method and was registered in Hungary in 1927. In the C_1 cycle 299 progeny were evaluated at one location by means of S_1 half-sib selection. The selection intensity was 5%. In the C_2 cycle 120 families were evaluated at two locations on the B 37 × A 632 tester and observations were made on the S_1s. The selection intensity was 7.5%. In the C_3 cycle there were 100 families, progeny testing was carried out at 2 locations with the tester CM105 × A 632 and observations were made on the S_1 generation. The selection intensity was found to be 10%. In the C_4 cycle 106 families were evaluated on the HMv AV-40 × HMv III-37 SLC tester and on hybrid NK PX 9283 SC at three locations, while observations were made on the S_1 at one location. Here the selection intensity was 12%.

The variety Mv Synthetic I was developed by Dr Márton Herczegh in 1974 by crossing 26 adapted lines. Among the lines 35% were Reid Y.D., mostly related to B 14, while the remainder were European dent lines. In the C_1 full-sib family selection was carried out on 300 families. Progeny testing was made at one location. The selection intensity was 4%. In the C_2 157 families were evaluated at two locations on the CM 105 × FR 19 SLC tester and observations were made on the S_1. The selection intensity was 6%. In the C_3 evaluations were made on 100 families at three locations using the FR 23 × FR 24 SLC tester and the S_1 was observed. A selection intensity of 14% was recorded. In the C_4 cycle 106 families were evaluated on the tester HMv 5330 × Co 255 at three locations and the S_1 was observed. The selection intensity was 15%.

A non-adapted, narrow-based population with good combining ability and 100% exotic blood was developed by crossing two different S_2 families from the same population of the variety Westigua, originating from Chile. In the C_1 cycle *per se* evaluations were made on 598 S_1 families at one location. A selection intensity of 2% was recorded. In the C_2 82 S_1 families were evaluated on the A 654 tester at two locations. The selection intensity here was 15%. In the C_3 cycle 100 S_1 families were evaluated on tester A 665 × HMv 16 SLC at three locations, giving a selection intensity of 10%. In the C_4 cycle 105 families were tested on NK PX 9283 SC at three locations and observations were made on the S_1 families. The selection intensity proved to be 15%.

Between 1976 and 1988 selection in Martonvásár was carried out at a plant density of 100,000 plants/hectare and the evaluation of the hybrids at 80,000 plants/ha. Crossing between the families chosen for the new cycle was achieved by sowing a mixture of 40 seeds from each of the chosen families. An inter-cross was carried out by crossing each row with the next row, the last being crossed with the first.

For the evaluation, the seed reserves of each cycle of the various populations were renewed in 1988. In 1989 the C_0–C_4 cycles of all three populations were crossed with testers HMv 124-2 and A 632. Due to seed setting deficiencies the complete genetic series could not be produced on line A 632, so the crosses developed on this line were excluded from the evaluation. In 1990 and 1991 the experiments were set up in a split-plot design at two locations. In each year one of the experiments was destroyed by bad weather, so evaluations were made on the results achieved in Martonvásár in 1990 and in Bácsbokod in 1991.

Results and discussion

Data on the effect of recurrent selection on the grain yield, grain moisture at harvest and percentage stalk lodging are presented in Table 1. Averaged over the two years a total grain yield of 32.8% was achieved over the course of 4 cycles for variety MYD, 35.6% for Mv Syn I and 19.7% for Westigua, with increases per cycle of 8.2%, 8.9% and 4.9%, respectively. This increase is slightly greater than that reported in the literature, though similar results have often been obtained in the first 3–4 cycles. Selection gain declines if the total increase is investigated over 8–10 cycles.

Table 1

Effect of recurrent selection on certain plant characters on the tester HMv 124-2, averaged over 2 years

Tester × population	Cycles	Yield average (t/ha)	Grain moisture at harvest (%)	Stalk lodging (%)
HMv 124-2 × MPS	C_0	7.98	26.1	6.2
	C_1	8.39	23.3	9.8
	C_2	8.79	23.5	9.5
	C_3	9.71	23.0	3.5
	C_4	10.60	24.1	6.2
$LSD_{5\%}$		0.24	0.53	3.2
HMv 124-2 × Mv Syn. I.	C_0	8.04	25.5	4.6
	C_1	8.64	26.8	2.9
	C_2	9.00	25.5	3.5
	C_3	10.11	24.2	3.6
	C_4	10.90	24.5	6.3
$LSD_{5\%}$		0.37	0.49	NS
HMv 124-2 × Westigua	C_0	9.02	26.0	3.0
	C_1	9.41	28.4	4.4
	C_2	9.74	25.0	3.7
	C_3	10.12	25.4	6.1
	C_4	10.80	24.6	2.7
$LSD_{5\%}$		0.27	0.68	NS

NS: non-significant

For the populations tested, the differences between the various cycles were similar, suggesting that economical population improvement could still be achieved over a further 1–2 cycles.

The grain moisture content decreased slightly but significantly in all three populations, by 0.5%/cycle in MYD, 0.25%/cycle in Mv Syn. I and 0.35%/cycle in Westigua. This finding is in agreement with data in the literature. No trend could be observed in changes in percentage stalk lodging in any of the populations. This means that the increase in yield was not achieved at the expense of an increase in vegetation period or a reduction in stalk strength, but by an increase in the frequency of gene combinations with a positive effect on yield. This was no doubt promoted by the fact that in all three populations plants with two or more ears were chosen when selecting both the S_0 plants and the S_1 families.

Averaged over 2 years and 3 populations, recurrent selection allowed certain generalisations to be made (Table 2). By means of recurrent selection an increase in yield potential of approx. 30% was achieved over the 4 cycles, averaged over the three populations, with a 1.5% reduction in grain moisture and no change in the percentage stalk lodging. Compared with the commercial standard, the yield in the 4th cycle was 87.9%, while that of the C_0 cycle was only 68.1%.

The inbreeding of S_1 families arising from the tested populations or from other population improvement programmes was continued. If the homozygous lines produced satisfied even the minimum seed production requirements they were tested with a wide range of lines of commercial value in order to develop commercial hybrids.

The results are summarised in Table 3. Of approximately 11,500 tested S_1 families originating from 115 populations only two homozygous lines have reached the stage where hybrids developed from them could be entered for registration trials, but none of these has become a registered commercial hybrid due to the unsatisfactory yield. Since the population improvement programme did not result in any inbred lines of commercial value even after a long period, both population improvement and the development of lines from the populations have been terminated.

Table 2

Effect of four cycles of recurrent selection on certain plant characters, averaged over 3 populations and 2 years on the tester HMv 124-2

Cycles	Yield average (t/ha)	Grain moisture at harvest (%)	Lodging resistance error (%)
C_0	8.35	25.9	4.6
C_1	8.81	26.1	5.7
C_2	9.18	24.6	5.6
C_3	9.98	24.2	4.4
C_4	10.77	24.4	5.2
$LSD_{5\%}$	0.17	0.30	NS

NS: non-significant

Table 3
Number of elite lines developed by population improvement

Selection cycle	No. of populations	Approximate number of S_1 families developed	No. of elite lines developed	No. of registered hybrids
C_0	55	5500	2	0
C_1	24	2400	0	0
C_2	24	2400	0	0
C_3	6	600	0	0
C_4	6	600	0	0
Total:	115	11500	2	0

This result is by no means surprising, since surveys by Bauman (1981), Duvick (1981) and Hallauer and Miranda (1981) have shown that almost all maize breeders have attempted to improve populations, but according to the compilation published by Gerdes et al. (1994), with the exception of B 14, B 37, B 73 and B 84, no lines of commercial value have been developed by means of population improvement. Strictly speaking only lines B 73 and B 84 originate from population improvement, since B 14 and B 37 were derived from the C_0 cycle.

There are no doubt many as yet uninvestigated reasons for the lack of success. Observations indicate that two of the most frequent reasons are:

1. The difference between the performance of commercial hybrids and source populations is still so great, even after successful selection, that the proportion of S_1 families which equals or exceeds the performance of the commercial hybrids in test crosses is extremely low;

2. The favourable agronomic properties of lines derived from populations segregate in the sublines, so there is little chance of developing lines which can be profitably used for seed production.

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CONCEPT FOR LAND USE BASED ON REGULAR INUNDATION IN THE CONTEMPLATED FLOOD STORAGE AREAS ABOVE SZEGED (HUNGARY)

Z. FODOR

INSTITUTE OF ENVIRONMENTAL MANAGEMENT, SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY

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The planned improvements on the Vásárhelyi plan propose the setting up of flood storage areas along the banks of the Hungarian section of the River Tisza. Two main options have been outlined to transform the land-use system of these areas. According to the first option, the present land-use system with a dominant ratio of arable lands would remain and land-users would receive compensation if the land was used for flood control. The second option suggests a change in the structure of land use to establish a system involving regular inundation in these areas. In this case not only extremely high water levels but all floods would be led out onto these flood storage areas. One of the most important conditions for the introduction of this scenario is the flood regime of the River Tisza.

This paper analyses the characteristics of this flood regime as it affects the planned flood storage area to be built near Szeged in southern Hungary on an area of approximately 6000 hectares between Baks, Ópusztaszer and Dóc. The statistics predict that the occurrence and duration of the floods could make the regular inundation of this area possible, but the fact that the water often recedes relatively rapidly may necessitate the partial retention of the floods.

Key words: wash-land utilisation, change of land use

Introduction

Improvements on the Vásárhelyi plan were prepared by the Water Resources Research Institute (VITUKI Rt.) on commission from the Ministry of Transport and Water Management in response to the extreme floods on the River Tisza between 1998–2001. It was concluded that there was a need to construct flood storage areas along the River Tisza to prevent extreme water levels in the case of future floods. These areas could be expected to be inundated once every 30 years.

Two scenarios were elaborated for the potential land use of these areas in the future. In the first scenario, the present land use system would generally remain in its current form. In the case of inundation, land users on these areas would receive compensation for the flood damage. According to the second scenario the land use of these areas would be fundamentally changed. The present 73.6% ratio of arable lands on these territories would be radically decreased to 5.5%, and the ratio of forests, meadows, pastures and non-intensive orchards would be increased. These land uses could utilise the more or less regular inundation, which would take place 6–7 years out of ten. In this case not only extreme floods, but all floods could be led out onto these areas. The primary function of these areas would be flood defence, and changes in land use should serve for the most rational utilisation of the new territorial characteristics (Szlávik, 2002).

Local initiatives emerging along the River Tisza support another approach, suggesting the renewal of certain elements in the type of land use practised before the river regulation in the 19th century, with the introduction of landscape management based on allowing flood water onto areas now protected from flooding. The primary aims of these initiatives are preservation, landscape rehabilitation and welfare, so they do not foresee allowing extreme floods onto these wash-lands, because water stagnation at a depth of several metres for several weeks would endanger the value of the newly created landscape.

Despite perceptible differences in the approach to extreme flood management, these two concepts, both projecting the inundation of currently flood-protected areas, suggest a similar transformation in the system of land use. The practicability of this change in land use will be limited by the flood regime of the river. There is little point in discussing this type of land use if regularly occurring floods do not provide adequate levels of water. The frequency of vernal and summer floods should primarily be taken into consideration, because it is mainly these that could be utilised by the desired land use system.

In the river system of the Tisza, vernal floods in March and April dominate, and this determines the expected period of inundation. Because of the accumulation of successive floods, there also tend to be floods in May on the river section below Szolnok. On the Upper Tisza floods are also frequent in June–July and November–December, but the high water levels generally subside below Tokaj and become insignificant on the Middle and Lower Tisza (Lászlóffy, 1982). The daily water levels recorded on the water-gauge in Szeged between 1876 and 1975 indicate the variability of the Tisza's water regime. In nearly forty percent of this period the river did not overflow its banks. A decade without floods is not unusual on the Tisza. Vernal floods only occurred in Szeged in half the years during this period, but summer floods were the most frequent here on the Hungarian section of the river (Vágás, 1982).

One of the planned flood storage areas would be created in a currently flood-protected basin between Baks, Ópusztaszer and Dóc above Szeged (Fig. 1).

The lowest parts of the flatland basin are less than 77 metres above the Baltic Sea level, while the highest points are over 80 metres. The rehabilitation of the former wash-land landscape is projected by the Csongrád County Association for Nature Protection, Szeged, who plan inundation for April or May, up to a level of 78 m. The flood would be retained in the basin for approximately a month, after which it would be sluiced into the Tisza (Paulovics, 2002).

The theoretical feasibility of this change in land use structure could be estimated by examining the water levels observed on this stretch of the river against the background of the general characteristics of the Tisza.

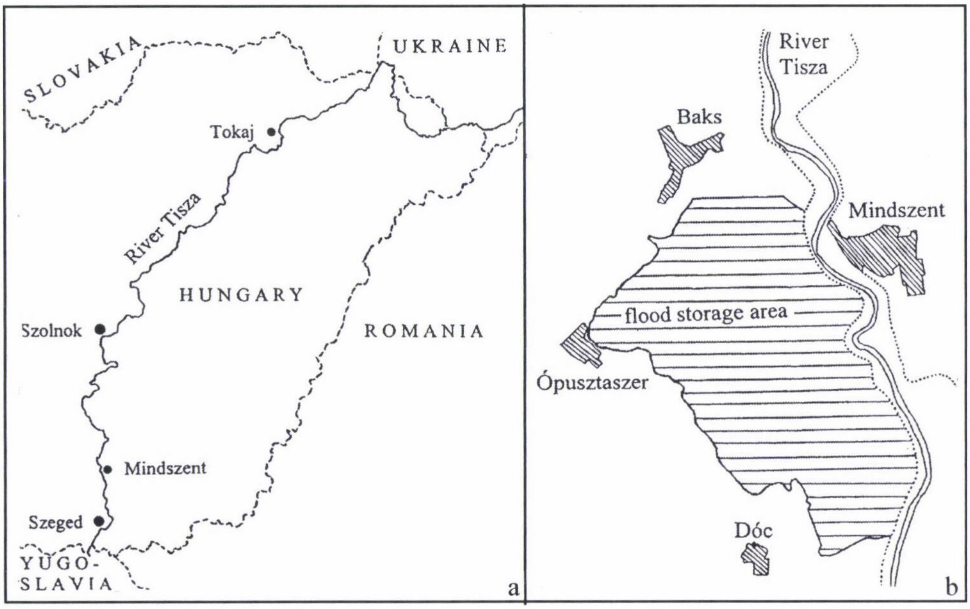

Fig. 1. The Hungarian section of the river Tisza and the planned flood storage area above Szeged

Materials and methods

Data from the water-gauge in Mindszent, situated at the 217.7 river-kilometres mark on the left bank of the river, opposite the Baks – Ópusztaszer – Dóc basin, were used to describe the water levels in the area. The height of the zero point on the gauge is 74.82 m above Baltic Sea level. The data of daily water levels between 1960 and 1999 were collected from the database of the Water Resources Research Centre.

The first step in the analysis was to construct a graph illustrating the relative frequency of various water levels in order to determine whether a water level of at least 78 m was reached with a frequency of at least 50%, or preferably 75% during the period investigated.

The forty values belonging to each day of the year were arranged in increasing order of magnitude to construe the graph. Values recorded on 29th February in leap-years were ignored. From the arranged data the first (minimum), the tenth, the twentieth and the fortieth (maximum) elements were plotted on the graph (Fig. 2).

This graph displays construed curves and does not disclose the real occurrence of water levels reaching the intended inundation height in various years. A further survey was made to reveal the actual frequency of adequate water levels during this period. Data recorded between April and June were highlighted for special study because of the importance of vernal and early summer floods for the projected land use. The analysis showed how many days the water level reached the 77, 78, 79 and 80 m height during these months in the period examined. The monthly values belonging to each water level were then arranged in decreasing order. From the arranged sequences of data, the first (maximum), the tenth, the twentieth, the twenty-fourth, the thirtieth, the thirty-sixth and the fortieth (minimum) terms were selected and arranged in Table 1. Data belonging to a water level of 78 m and to relative frequencies between 50 and 75% have been highlighted in the table.

Future changes in the water levels serving as the basis of alternative land use can be predicted by determining the number of years in the period surveyed when the water level remained at 78 metres for at least 5, 7, 10, 15 or 20 days during the months of April to June. The standard deviations for these figures were then determined. Since the relative frequency (p) shows a binomial distribution with an expected value of $k = n \times p$ (where n is the total number of cases and k the number of actual cases), the deviation (σ) can be calculated with the following formula:

$$\sigma = \sqrt{n \cdot p \cdot (1-p)}$$

(Vágás, 1982).

On this basis the $k \pm 2\sigma$ intervals belonging to the actual case numbers were calculated, where the numbers of days were rounded up to an integer. Among the $k \pm 2\sigma$ intervals describing changes in the expected value (k) with an accuracy of 95.4%, those where the lower limit of the interval was not less than half of the total number of cases (n) were highlighted (Table 2).

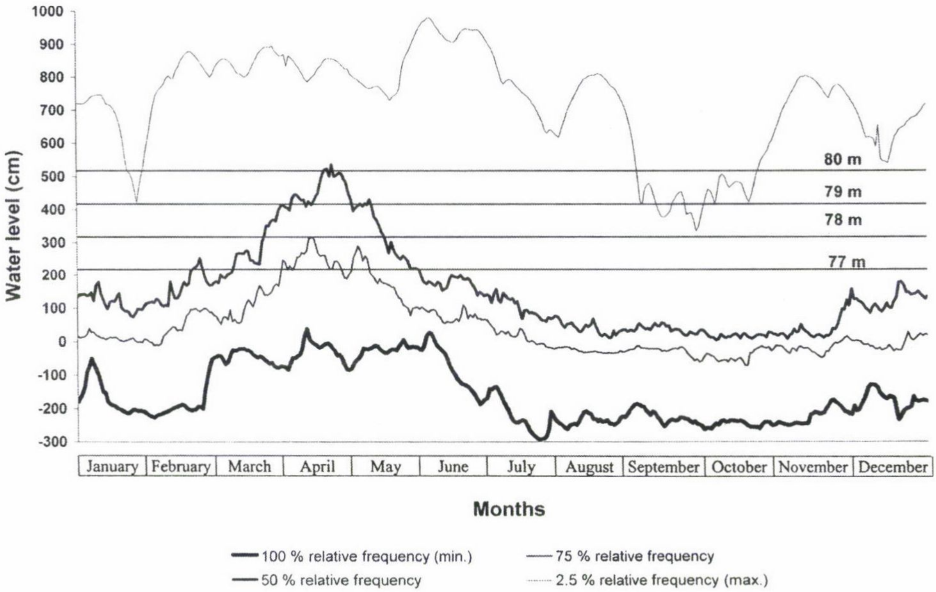

Fig. 2. Relative water level frequencies on the river Tisza as measured on the water-gauge in Mindszent between 1960 and 1999 (217.7 river km mark, 74.82 m)

Table 1

Relative frequency (%) of water levels above 77, 78, 79 and 80 metres above sea level as measured on a water-gauge in Mindszent between 1960 and 1999

Relative	April (day/month)				May (day/month)				June (day/month)				Whole year (day/year)			
frequency	77 m	78 m	79 m	80 m	77 m	78 m	79 m	80 m	77 m	78 m	79 m	80 m	77 m	78 m	79 m	80 m
2.5	30	30	30	30	31	31	31	31	30	30	30	30	261	236	201	159
25.0	30	30	29	27	31	24	17	12	21	13	6	0	176	129	101	73
50.0	30	25	20	10	22	13	9	1	12	4	0	0	131	85	57	35
60.0	28	20	11	3	16	9	4	0	7	2	0	0	111	71	49	24
75.0	21	11	0	0	12	7	1	0	0	0	0	0	90	67	41	14
90.0	0	0	0	0	0	0	0	0	0	0	0	0	30	9	0	0
100.0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0

Table 2
Number of years (k) in which water levels reached 78 m for the given number of days per month, as measured on a water-gauge in Mindszent between 1960 and 1999

Number of days on which water levels reached 78 m	April		May		June	
	k	$k \pm 2\sigma$	k	$k \pm 2\sigma$	k	$k \pm 2\sigma$
5	34	29–39	33	28–38	20	14–26
7	33	28–38	31	26–36	17	11–23
10	31	26–36	24	18–30	15	9–21
15	26	20–32	19	13–25	10	5–15
20	25	19–31	14	8–20	8	3–13

Results

On the graph constructed from data series taken from the water-gauge in Mindszent very low water levels of below –250 cm can be seen. These low levels occurred before the commissioning of the barrage in Törökbecse (Novi Bečej, Yugoslavia), and no levels below –80 cm have occurred since 1975. It can be seen from the graph that the planned 78 metres (318 cm) inundation level is slightly above the 75% relative frequency curve. The 50% relative frequency curve is above this level from March 23 to May 13.

According to the table showing the actual monthly occurrence of daily water levels, the water level reached the 78 m mark at a relative frequency of 75% on 11 days in April and 7 days in May, and at a relative frequency of 50% on 25 days in April and 13 in May. In June water levels at the intended inundation height were less frequent.

Since the range of deviation values is twice as high for the number of months in which the 78 m water level was reached for 5, 7, 10, 15 or 20 days, it can be expected with a certainty of 95.4% that at Mindszent this water level will be reached for 15 or more days in April and 7 or more days in May in half the years between 2000 and 2039.

The upper limits of the rather wide confidence intervals of 95.4% certainty are also able to show the months in which water levels are not expected to reach the planned inundation level for at least 5 days in at least half of the relevant period. In the present case there were no such months. On the basis of the confidence intervals it cannot be excluded that water levels of 78 m will be recorded between the years 2000 and 2038 for a minimum of 20 days in at least half of the Aprils, for a minimum of 15 days in half of the Mays, and for a minimum of 10 days in half of the Junes.

Discussion

The analysis confirms that, on the section of the river examined, the floods recede relatively early, in April, under regulated conditions, although the period with a tendency to drought extends into May.

The predicted frequency of water levels reaching 78 m in the area of the contemplated flood storage area above Szeged could be sufficient to guarantee the regular inundation of the lower-lying areas; however, since the floods recede relatively early and rapidly, the partial retention of the floods must also be provided for. Additional research is needed to determine whether the artificial supplementation of water or planned temporary periods of "dry" use will be the best type of management for this area in the case of protracted flood-free periods.

It can thus be concluded that through this transformation of land use a completely new system, potentially better suited to the natural circumstances, but definitely artificially created and maintained, could be established.

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RESPONSE OF TWO SUNFLOWER HYBRIDS TO PLANTING DATES AND DENSITIES

A. Y. ALLAM, G. R. EL-NAGAR and A. H. GALAL

DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE, ASSIUT UNIVERSITY, ASSIUT, EGYPT

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This investigation was carried out at the Experimental Farm of Assiut University during the summers of 2000 and 2001 to study the responses of two sunflower hybrids (Vidoc and Euroflora) to planting dates (May 1st, June 1st and July 1st) and planting densities (55,533, 83,300 and 166,600 plants/ha). The results indicated that the two varieties differed highly significantly in all studied traits except oil yield/ha. The highest seed yield (3.64 t/ha) was obtained with the variety Vidoc. In addition, the results revealed that the planting date exerted a highly significant influence on all vegetative growth traits along with yield and its components. Increasing plant density increased the seed and oil yield/ha. By contrast, the stem diameter, head diameter, 100-seed weight and seed yield/plant decreased with increasing plant density.

The interaction between varieties and plant density had a highly significant effect on head diameter. The greatest head diameter (20.06 cm) was recorded for the variety Vidoc planted at lower density. Concerning the interaction between planting density and planting date, the highest seed yield (4.47 t/ha) was obtained from dense plants at the early sowing date, and the highest oil % (45.32) at the late planting date and the lowest plant density.

The second order interaction exerted a highly significant influence on stem and head diameter in addition to seed yield/plant, where the highest value (78.13 g/plant) was obtained with the variety Vidoc planted on May 1st at the lowest plant density.

Key words: sunflower, planting dates, planting densities

Introduction

Sunflower is an important oil seed crop throughout the world. In Egypt, there is a severe shortage in edible oil production. The local production of oil does not exceed 15% of consumption (Osman, 2001). Therefore, increasing oil production must depend on the cultivation of new oil crops. Sunflower is considered one of the promising oil crops in Egypt, that could meet the oil needs. The area cultivated with sunflower increased from 6500 hectares in 1980 to 19,500 hectares in 2000 (FAO, 2000). There is a great need to improve the yield and quality of sunflower, which are important to farmers. Investigators who evaluated sunflower cultivars under Egyptian conditions (El-Mohandes and Kandil, 1986; Kandil and El-Mohandes, 1986; Kandil and Khalil, 1986) stated that the responses of introduced sunflower cultivars differed under Egyptian conditions. Keshta et al. (1993) studied the performance of three sunflower genotypes (Miak, HA89 and HM 77) at three planting densities, i.e. 41,650, 55,533 and 83,300 plants/ha. They indicated that the genotype HA89 produced the highest values for head diameter, seed yield/plant, 100-seed weight, seed oil content, seed and oil yield/ha, followed by Miak and HM77.

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El-Gharieb et al. (1996) indicated that the cultivar Miak significantly surpassed Hysun 354 and Elya in biological yield/ha and 100-seed weight. Moreover, Miak and Hysun 354 significantly exceeded Elya in seed and protein yields/ha. Sharief (1998) reported that the cultivar Phoebes significantly exceeded Pioneer and Cloforz in plant height, stem diameter, head diameter, 100-seed weight, seed yield/plant, oil %, and seed and oil yields/ha. Abul-Naas et al. (2000) and Basha (2000) found significant differences between sunflower varieties in number of seeds/head, plant height, seed index, seed and oil yields/ha.

Plant density plays an important role in sunflower productivity. El-Baz (1995) showed that an improvement was observed in plant height, stem diameter and number of green leaves/plant with each increase in hill spacing. Allam and Galal (1996) revealed that plant height and seed yield/ha increased with increasing plant density, while maximum values of head diameter and 100-seed weight were obtained when the plant population decreased. The same trends were generally reported by other investigators such as Salera (1998), Goksoy and Turan (1999) and Basha (2000), who noted that widening spaces between sunflower plants were reflected in a significant decrease in plant height, while the stem diameter, head diameter and seed weight/head increased with increasing plant spacing.

A delay in planting from April 15th to May 17th decreased plant height, stem and head diameters, number and weight of seeds/plant, seed yield/plant and seed oil content (Hussein et al., 1988; Sultan et al., 1988; Salamah et al., 1989).

El-Saied et al. (1989) found that the best sowing date for seed and oil yields was February 5th, followed by May 5th. Yakout et al. (1992) studied the responses of four sunflower genotypes, Miak, H7166, H7780 and H7107, to planting dates, namely the first of May, July and August. The best date for maximizing seed yield was May 1st and June 1st in the first and second seasons, respectively. On the other hand, seed oil content was not significantly affected by sowing date, as reported by Nandhagopal et al. (1995). In spring sowing, the seed yield was decreased with a delay in sowing from the beginning of February to the end of April (Sidhu et al., 1995; Kathuria et al., 1996).

The aim of this study was to investigate the response of two hybrids of sunflower to planting dates and densities.

Materials and methods

The trials were carried out at the Experimental Farm of the Faculty of Agriculture, Assiut University during the summer seasons of 2000 and 2001 to study the responses of two hybrids of sunflower (*Helianthus annuus* L.) to sowing dates and planting densities. The physical and chemical analyses of the soil of the experimental site are presented in Table 1.

A completely randomized block design with a split-split plot arrangement and four replications was used in both seasons. The experimental unit area was 3×3.5 m^2. The three sowing dates, May 1st (D_1), June 1st (D_2) and July 1st (D_3), were assigned to the main plots. The sub-plots were allocated to the sunflower hybrids, namely Vidoc (V_1) and Euroflora (V_2). Three planting densities (55,533 (P_1), 83,300 (P_2) and 166,600 (P_3) plants/ha) were obtained by planting on one side of 60 cm wide ridges with the hills 30, 20 and 10 cm apart, respectively.

All other cultural practices were carried out as recommended for sunflower production in both growing seasons.

At harvest, a random sample of 20 guarded plants per plot was taken and measurements were made on plant height (cm), stem diameter (cm), head diameter (cm), seed yield/plant (g) and 100-seed weight (g). The seed yields were recorded on a plot basis. The recorded values were used to estimate the corresponding values per hectare. Seed oil content was determined according to the procedure described by A.O.A.C. (1995). Oil yield (t/ha) was also calculated. Bartlett's test of variance homogeneity was carried out before the combined analysis of the two seasons according to Snedecor and Cochran (1980). Analysis of variance for all characters was carried out according to Gomez and Gomez (1984). The means were compared using L.S.D. at the 5% level.

Table 1

Physical and chemical characteristics of a representative soil sample from the experimental site

Determination	2000	2001
Sand (%)	26.60	24.65
Silt (%)	25.00	29.88
Clay (%)	48.40	49.50
Field capacity (%)	41.00	44.20
Organic matter (%)	1.83	1.58
ECe (dS/m)	1.95	1.46
pH 1:1 in water	7.40	7.88
Calcium carbonate (%)	3.70	3.18
Total nitrogen (%)	0.14	0.16

Results and discussion

Bartlett's test of homogeneity indicated that the variance of the data of both seasons was insignificant. Thus, combined analysis was carried out and the average of the two seasons is presented in the tables.

Vegetative growth traits

The data in Table 2 revealed that there were highly significant differences between the sunflower varieties in plant height, stem diameter and head diameter. It is clear from these data that Vidoc plants were taller with a greater head diameter as compared to Euroflora, while the shorter plants of Euroflora were accompanied by thicker stalks.

These differences between the varieties may be due to differences in the response of the plants to the environmental factors prevailing during growth. These results are in line with those obtained by Allam and Galal (1996), El-Gharieb et al. (1996), Abul-Naas et al. (2000) and Basha (2000).

Table 2

Effect of sowing dates and plant densities on yield, its components and quality over two growing seasons for two sunflower hybrids

Treatments		Plant height, cm	Stem diameter, cm	Head diameter, cm	100-seed mass, g	Seed yield/plant, g	Seed yield, t/ha	Oil %	Oil yield, t/ha
Varieties									
Vidoc	V_1	142.15	1.09	18.14	7.21	62.70	3.64	41.17	1.50
Euroflora	V_2	139.45	2.03	16.75	6.74	57.04	3.38	42.00	1.43
F-test		**	**	**	**	**	**	**	N.S.
Planting densities (plants/ha)									
55,533	P_1	134.02	2.14	19.17	7.33	61.19	2.98	41.74	1.24
83,300	P_2	141.70	1.98	17.52	6.63	54.01	3.52	42.30	1.48
166,600	P_3	146.67	1.86	15.65	6.98	51.46	4.07	40.70	1.64
F-test		**	**	**	**	**	**	**	**
L.S.D.		1.08	0.058	0.23	0.193	0.920	0.07	0.15	0.06
Planting dates									
May 1st	D_1	122.87	2.01	20.14	7.42	68.66	3.88	38.87	1.50
June 1st	D_2	153.68	2.10	17.32	6.96	59.74	3.45	41.23	1.43
July 1st	D_3	145.84	1.87	14.99	6.55	51.21	3.24	44.65	1.43
F-test		**	**	**	**	**	**	**	N.S.
L.S.D.		1.26	0.05	0.076	0.11	1.23	0.07	0.30	–

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively. N.S. = Not significant.

The data revealed that sowing date exerted a highly significant influence on all vegetative growth traits. It is clear from these data that plants sown on June 1st were the tallest (153.68 cm) as compared to those sown on the other two dates. These plants also had the greatest stem diameter (2.10 cm). Plants sown on May 1st had the highest value of head diameter (20.14 cm) when compared to the other sowing dates, especially those sown on July 1st, where the lowest value (14.99 cm) was obtained. These results are in harmony with those obtained by Keshta et al. (1993), El-Gharieb et al. (1996), Abul-Naas et al. (2000) and Basha (2000).

The data in Table 2 showed that different vegetative traits were significantly affected by plant populations. The denser-sown plants produced taller plants as compared to the lower plant densities.

A decrease in the plant population per unit area may increase light penetration within the plant canopy and consequently decrease stem elongation, since an increase in light intensity exerts an effect on the auxin balance within the plants and reduces the plant height. Plants grown with low plant density thus become thicker as compared to more densely sown plants. The reduction in plant height and the increase in stem thickness could lead to more assimilates being available for head formation. Similar results were reported by El-Saied et al. (1989), Kandil and Khalil (1986), Keshta et al. (1993) and Salera (1998).

Due to competition for light, dense plants require more photosynthesis products to grow in height and less resources will be devoted to heads and seed formation.

The data showed that vegetative traits were highly significantly affected by the interaction between date of sowing and varieties. The tallest plants with greater stem diameter were obtained from Vidoc when sown on June 1st. On the other hand, the highest value of head diameter (20.18 cm) was obtained from Vidoc when sown on May 1st (Table 3).

Also, the interaction between varieties and plant population exerted a highly significant influence on head diameter. The largest head diameter (20.06 cm) was obtained from Vidoc when sown at wider spacing (low density plants).

The interaction between population density and planting date exerted a highly significant influence on stem and head diameter, where the highest value of stem diameter (2.33 cm) was obtained from planting on June 1st at the lowest plant density. On the other hand, the highest value of head diameter (21.67 cm) was obtained from planting on May 1st with the lowest plant density.

Table 3

Effect of first order interactions between sowing dates, planting densities and two hybrids of sunflower on yield, its components and quality over two growing seasons

Treatments		Plant height, cm	Stem diameter, cm	Head diameter, cm	100-seed mass, g	Seed yield/plant, g	Seed yield, t/ha	Oil %	Oil yield, t/ha
	P_1	135.67	2.07	20.06	7.52	69.30	3.12	42.22	1.29
V_1	P_2	142.75	1.97	18.03	7.21	61.97	3.67	40.75	1.55
	P_3	148.95	1.84	16.34	6.91	56.82	4.19	40.53	1.62
	P_1	145.33	2.21	18.28	7.13	62.99	2.83	42.72	1.31
V_2	P_2	140.64	1.99	17.01	6.75	56.95	3.36	42.06	1.50
	P_3	132.37	1.89	14.97	6.34	51.20	3.95	41.22	1.69
F-test		N.S.	N.S.	**	N.S.	N.S.	N.S.	*	*
L.S.D.		–	–	0.30	–	–	–	0.21	0.10
D_1	V_1	123.99	2.11	20.18	7.60	70.00	4.00	39.09	1.52
	V_2	121.76	1.90	19.89	7.24	67.32	3.76	38.65	1.45
D_2	V_1	161.35	2.15	18.94	7.33	66.26	3.59	40.65	1.48
	V_2	146.01	2.05	15.70	6.59	53.23	3.28	41.81	1.38
D_3	V_1	148.25	1.92	15.31	6.77	51.84	3.36	43.76	1.48
	V_2	143.33	1.82	14.67	6.34	50.58	3.09	45.54	
F-test		**	**	**	**	**	N.S.	**	N.S.
L.S.D.		2.145	0.086	0.15	0.31	1.11	–	0.38	–
	P_1	116.33	2.33	17.98	7.90	76.28	3.21	39.77	1.29
D_1	P_2	124.06	2.04	20.45	7.24	67.40	4.36	39.09	1.52
	P_3	128.23	1.93	21.67	7.12	62.30	4.47	37.76	1.67
	P_1	147.08	2.07	15.87	7.22	65.40	3.52	41.82	1.21
D_2	P_2	154.90	2.03	17.04	6.99	59.85	3.24	41.35	1.48
	P_3	159.05	1.92	19.05	6.67	53.97	3.74	40.52	1.57
	P_1	138.65	2.01	13.39	6.94	56.75	2.95	45.32	1.24
D_3	P_2	146.13	1.87	15.10	6.63	51.13	3.40	44.79	1.45
	P_3	152.74	1.74	16.48	6.09	45.75	3.95	43.84	1.62
F-test		N.S.	**	**	*	**	**	**	N.S.
L.S.D.		–	0.01	0.392	0.33	1.59	0.05	0.25	–

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively. N.S. = Not significant. For treatment details, see Table 2.

The second order interaction (Table 4) exerted a significant influence on all growth traits except plant height. In this respect again, a decrease in the measured traits was associated with dense planting. This revealed that increasing competition due to high population reduced the stem diameter and head diameter due to the partitioning of assimilates towards longer stems. This trend was similar in both varieties and at all planting dates. This also means that density is the major factor affecting the significant traits of sunflower under the experimental conditions. Similar findings were reported by El-Baz (1995), Nandhagopal et al. (1995) and Sharief (1998).

Seed yield and yield components

It is clear from the data in Table 2 that the variety exerted a highly significant influence on yield and yield components, since Vidoc hybrid plants had higher seed index, seed yield/plant and consequently seed yield/ha. The increase in seed yield/plant may be due to the increase in head diameter, which may include an increase in the number and size of seeds/head.

Table 4

Effect of second order interactions between sowing dates, planting densities and two hybrids of sunflower on yield, its components and quality over two growing seasons

Treatments			Plant height, cm	Stem diameter, cm	Head diameter, cm	100-seed mass, g	Seed yield/plant, g	Seed yield, t/ha	Oil %	Oil yield, t/ha
D_1	V_1	P_1	116.11	1.83	22.86	7.91	78.13	3.33	39.86	1.33
		P_2	122.13	2.02	20.37	7.45	68.33	3.97	39.57	1.81
		P_3	127.03	1.84	17.91	7.28	63.54	4.62	37.88	1.67
	V_2	P_1	116.55	2.29	21.69	7.73	74.44	3.18	39.68	1.24
		P_2	125.99	2.05	20.49	7.18	66.47	3.74	38.75	1.45
		P_3	129.42	2.00	17.49	6.96	61.06	4.38	37.63	1.67
D_2	V_1	P_1	155.20	2.41	21.17	7.58	73.31	3.14	41.32	1.29
		P_2	162.10	2.02	18.53	7.32	65.91	3.57	40.85	1.57
		P_3	166.75	2.01	17.11	7.09	59.73	4.09	39.77	1.57
	V_2	P_1	138.96	2.24	16.93	6.86	57.63	2.62	42.33	1.17
		P_2	147.71	2.06	15.54	6.68	53.80	3.26	41.85	1.38
		P_3	151.36	1.85	14.63	6.26	48.22	3.83	41.26	1.57
D_3	V_1	P_1	135.70	1.94	16.74	7.06	56.64	2.83	44.50	1.26
		P_2	144.02	1.87	15.19	6.87	51.68	3.86	42.91	1.62
		P_3	150.27	1.66	13.99	6.87	47.20	3.86	42.91	1.62
	V_2	P_1	141.60	2.08	16.22	6.81	56.96	2.62	46.14	1.19
		P_2	148.34	1.84	15.00	6.40	50.53	3.07	45.69	1.40
		P_3	155.21	1.82	12.78	5.81	44.30	3.62	44.78	1.62
		F-test	N.S.	**	**	N.S.	**	N.S.	N.S.	N.S.
		L.S.D.	–	0.14	0.56	–	2.26	–	–	–

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively. N.S. = Not significant. For treatments details, see Table 2.

Planting date exerted a significant influence on yield and yield components. The results showed that sowing earlier (May 1st) produced the highest seed yield/ha (3.88 t) as compared to the other two planting dates, especially when seeds were sown on July 1st, when the lowest yield (3.24 t) was obtained. The increase in yield in early-sown plants was mainly due to the increase in seed index and seed yield/plant, the increase in the latter being mainly due to the increase in head diameter. The increase in seed yield/ha after the May 1st sowing was 12.40 and 19.85% as compared to that obtained on June 1st and July 1st, respectively.

The increase in yield after early planting may be due to the increase in the length of the growing season, which led to the production of more assimilates through photosynthetic processes, with a consequent increase in seed size and number, leading to an increase in seed yield/plant. These findings are in harmony with those reported by Yakout et al. (1992), Allam and Galal (1996), Kathuria et al. (1996) and Sharief (1998).

For plant population, the data revealed that plant density had a highly significant influence on yield and yield components. The data showed that the highest seed yield per hectare (4.07 t) was obtained when sunflower plants were sown at higher plant population (166,600 plant/ha), the increase here being mainly due to the increase in number of plants at harvest. By contrast the data showed that the highest seed index and seed yield per plant were obtained from wide spacing (less dense population), due to the increase in head diameter. The lowest yield per plant, obtained at high plant density (166,600 plants/ha), could be due to the reduction in seed index and seed yield/plant. The competition between plants at high density for light and other environmental factors was high, forcing the plants to grow taller with more vegetative growth at the expense of seed production. The present results confirm the findings of Salamah et al. (1989), Yakout et al. (1992), Kathuria et al. (1996) and Abul-Naas et al. (2000).

The interaction between planting dates and varieties exerted a significant influence on yield and yield components except for yield/ha. The highest values were obtained with Vidoc when sown on May 1st (Table 3).

It is important to note that the superiority of Vidoc was clear at all planting dates. This means that the genetic make-up of Vidoc is the predominant factor in this interaction.

Furthermore, the interaction between planting dates and plant populations had a significant or highly significant influence on all yield traits. The highest values of seed index and seed yield/plant were obtained by planting on May 1st with the lowest plant density, due mainly to the higher vegetative growth attributed to these interactions. On the other hand, the highest seed yield/ha was obtained by planting on May 1st with moderate or high plant density. For this trait, it seemed that the number of plants compensated for the low value of yield/plant leading to a high value of yield/ha (Table 3).

The second order interaction exerted a significant influence on seed yield/plant, where the highest value (78.13 g) was obtained from the hybrid Vidoc when sown on May 1st with the lowest plant density. In all the interaction combinations, low density plants produced higher plant yield due to larger heads and higher seed index values. Furthermore, the pattern of response was similar in both tested varieties (Table 4). These results are in line with those reported by Salamah et al. (1989), Yakout et al. (1992), Sharief (1998) and Basha (2000).

Oil % and oil yield/ha

The data in Table 2 showed that the variety exerted a highly significant influence on the oil % but had no significant effect on the oil yield/ha. It is clear from the results that seeds of the hybrid Euroflora had more oil (42.00%) than those of Vidoc (41.17%).

Despite the non-significant differences between the varieties, Vidoc had a slightly greater oil yield than Euroflora. The increase was mainly due to the increase in seed yield/ha rather than to oil %. The same trend was reported by El-Saied et al. (1989), Keshta et al. (1993), El-Gharieb et al. (1996) and Basha (2000).

The data revealed that sowing date exerted a highly significant influence on oil %. It is clear from these data that seeds from the late planting treatment contained more oil (44.65%) as compared to the other sowing dates, especially from plants sown on May 1st, where the lowest value was obtained (38.87%). The increase in oil % in late-sown plants may be due to the fact that the increase in temperature during seed development in late-sown plants led to an increase in the synthesis of oil in the seeds.

By contrast, the highest oil yield (1.50 t) was obtained from plants sown at the earliest date (May 1st), due to the increase in seed yield/ha, since the highest seed yield was also obtained from sowing on May 1st. Similar results were reported by Kathuria et al. (1996) and Abul-Naas et al. (2000).

The data also showed that the plant population exerted a highly significant influence on oil % as well as oil yield/ha. It is clear from these data that the lowest oil yield/ha (1.24 t) was obtained from plants sown at low density. This was due to the reduction in seed yield, despite the increase in oil % (41.74). While the highest oil yield/ha (1.64 t) was obtained from the densest plants (166,600 plants/ha), this was mainly due to the increase in seed yield (4.07 t/ha). These results are in general agreement with those obtained by Salamah et al. (1989), Yakout et al. (1992) and Sharief (1998).

It is clear from these data that the oil % was significantly affected by the interaction between varieties and sowing dates (Table 3), where the highest value (45.54%) was obtained from the Euroflora hybrid when sown at the latest date (July 1st). This variety was superior to Vidoc at all sowing dates.

The interaction between varieties and plant populations also exerted a significant influence on oil % and yield, the highest oil % value (42.72%) being obtained from the hybrid Euroflora when sown at low density. On the other hand, oil % was significantly affected by the interaction between sowing dates and plant populations, the highest value (45.32%) being obtained from sowing on July 1st with the highest plant density.

The oil yield was significantly affected by the interaction between varieties and plant populations, the highest value being obtained from Vidoc or Euroflora when sown at moderate population density. The second order interaction had no significant effect on oil % and oil yield/ha.

The present investigation revealed that planting dates, densities and varieties had significant effects on growth characters, seed yield, yield components, and the oil percentage and yield. These factors should be taken into consideration when planting sunflower for maximum seed and/or oil yields/hectare under Assiut conditions.

Conclusions

The data in Table 5 revealed that the simple correlations between all the traits studied were significant, except for the correlation between plant height and stem diameter.

It could be concluded from this investigation that planting the hybrid Vidoc on May 1st with a plant density of 166,600 plants/ha produced the highest yields of seed and oil under Assiut conditions.

Table 5

Simple correlation between the studied traits of sunflower

Plant characters	Plant height	Stem diameter	Head diameter	100-seed mass	Seed yield /plant	Seed yield/ha	Oil %	Oil yield
Oil yield	–0.242**	0.176*	0.389**	0.172**	0.328**	0.788**	–0.214**	–
Oil percentage	0.481**	–0.322**	–0.758**	–0.330**	–0.719**	–0.627**	–	
Seed yield/ha	–0.420**	0.219**	0.639**	0.272**	0.572**	–		
Seed yield/plant	–0.490**	0.501**	0.935**	0.371**	–			
100-seed mass	–0.475**	0.303**	0.790**	–				
Head diameter	–0.558**	0.473**	–					
Stem diameter	–0.059	–						
Plant height	–							

*, ** Significant and highly significant at the 1% and 5% levels of probability.

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EFFECTS OF CASSAVA GENOTYPE, CLIMATE AND THE *BEMISIA TABACI* VECTOR POPULATION ON THE DEVELOPMENT OF AFRICAN CASSAVA MOSAIC GEMINIVIRUS (ACMV)

I. ABDULLAHI[1,2], G. I. ATIRI[1], A. G. O. DIXON[2], S. WINTER[3] and G. THOTTAPPILY[2]

[1]DEPARTMENT OF CROP PROTECTION AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF IBADAN, IBADAN, NIGERIA
[2]INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE, IBADAN, NIGERIA
[3]DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN, BRAUNSCHWEIG, GERMANY

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A survey was carried out in the 1996/97 and 1997/98 growing seasons on a field planted in three replicates with five clones of cassava at the International Institute of Tropical Agriculture, Ibadan, located in a transition forest, to determine the effects of cassava genotype and climate on the development of African cassava mosaic geminivirus (ACMV) and changes in the *Bemisia tabaci* population. Cassava genotype, climate and their interactions have significant ($P<0.01$) effects on the population of *B. tabaci* and the development of ACMV. The incidence of ACMV was significantly ($P<0.01$) higher in clones 81/01635 and 92/0520 than in TMS 30572 and 94/0239, while 91/02327 showed the greatest resistance. A positive correlation between the incidence and severity of ACMV was observed, but this did not correlate with the whitefly population density.

Key words: ANOVA, *geminivirus*, incidence, *Manihot esculenta*, severity, resistant variety, whitefly

Introduction

Cassava, *Manihot esculenta* Crantz, is an important food crop in Africa, providing more than 50% of the caloric requirement for more than 200 million people on the continent (FAO, 1997). Although it thrives better than other crops in poor soils, a large gap exists between the potential yield (24 t/ha) and that realized by farmers (8.51 t/ha) (FAO, 1999). African cassava mosaic geminivirus (ACMV) is the main biotic constraint on cassava production and the most important threat to food security in sub-Saharan Africa (Guthrie, 1987). ACMV is vectored by *Bemisia tabaci* in a persistent manner (Dubern, 1979).

The incidence of ACMV could be as high as 100% (Walker et al., 1985) and its severity varies significantly with cassava cultivar (Njock et al., 1996), soil conditions and plant age (Hahn et al., 1989; Fargette et al., 1994). The infection pressure of ACMV, which has been viewed as the rate of spread, is generally high in rainforest areas and low in the savanna (Fauquet et al., 1988). An epidemic outbreak of ACMV (Otim-Nape et al., 1997), which was not attributed to changes in agroecological or environmental conditions, has led to speculations of a possible occurrence of a new strain of *B. tabaci* (Gibson et al.,

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1996). However, the epidemic was observed to be associated with an increased abundance of *B. tabaci* (Legg, 1995), and later attributed to a synergistic interaction between *B. tabaci* and virus-infected cassava host plants (Colvin et al., 1999).

It is important to have a thorough understanding of the various components in a virus pathosystem, in order to develop sustainable control strategies (Atiri et al., 2000). The effect of climate on the ecology of ACMV is multifarious, affecting the vector (Fishpool and Burban, 1994; Fishpool et al., 1995), the host plant, and also other pests and diseases of cassava, which interact with ACMV (Otim-Nape, 1993). In addition to climate, the cropping system also affects the activities of *B. tabaci* (Ahohuendo and Sarkar, 1995).

Breeding for resistance has been considered a feasible strategy for the control of ACMV (Guthrie, 1987). However, the performance of resistant varieties can only be guaranteed under certain conditions. Increased inoculum pressure, agroecological changes, vector population explosion or immigration of a new vector biotype can have a significant impact on the expression of resistance. Five cassava varieties, developed by the International Institute of Tropical Agriculture (IITA), which are being screened for multigenic resistance, were selected for this study to determine the effect of cassava genotype on ACMV development, *B. tabaci* population and the host plant-virus-vector interactions. The role of climatic factors in the virus-vector relationship in the transition rain forest was also investigated.

Materials and methods

Plant materials and insect population monitoring

The population of *B. tabaci* was monitored in the field at IITA, Ibadan. Five clones of cassava were used: TMS 30572, TMS 91/02327, TMS 81/01635, TMS 92/0520 and TMS 94/0239, which were developed by IITA and are being screened for multiple pest resistance. Each clone was planted at a distance of 1 m × 1 m, in a plot measuring 5 m × 10 m. There were three replicates of each clone.

The counting of insects started in April 1996, three weeks after planting. Counting was done every two weeks for two growing seasons, 1996/97 and 1997/98. The direct count method (Fargette et al., 1985) was used. Counting was done in the morning, between 7.00 a.m. and 8.00 a.m. Six cassava stands were randomly selected per plot as representative samples; one of these was made a reference sample.

Virus, vector, host and climate

The incidence and severity of ACMV were monitored for the same period and time interval as described above. Incidence was determined by expressing the number of infected plants in a plot as a percentage of the total number of plants within the plot. For severity assessment, six stands of cassava were randomly selected from infected plants as representative samples for the plot, as described earlier. The proportions of the leaf tissue showing symptoms were scored on a 0–4 scale. Temperature, relative humidity and rainfall data were obtained from the IITA meteorological station. Two weekly averages of temperature and relative humidity were used to determine the effect on the *B. tabaci* population and the incidence and severity of ACMV. For the determination of rainfall effects, two weekly totals were used. These two weekly data corresponded with the time of field data collection.

Data analysis

Disease incidence was subjected to Arcsine transformation, whereas severity was subjected to logarithmic transformation before statistical analysis (SAS, 1989). The data for relative humidity were subjected to square root transformation. Rainfall data were subjected to log transformation, whereas temperature data were used without transformation since variation here was not significant. The repeated measures analysis version of analysis of variance (ANOVA) (SAS, 1989) was performed on the adult population as a function of cassava genotype and age of the plant from date of planting. This type of analysis was recommended since data were obtained by repeated measurements on the same experimental unit (Ende, 1993). Before analysis, the adult population was subjected to square root transformation. Duncan's (1955) multiple range test was used for mean separation at the 0.01 level of significance.

Results

Effect of cassava clones on the Bemisia tabaci *population*

The population of *B. tabaci* was found to vary significantly ($P<0.01$) among the five cassava clones and with the age of the cassava plants. A significant ($P<0.01$) interaction between plant age and variety was also observed. Clone 91/02327 supported the highest number of insects and, in the multiple range test, the population on this clone was found to be significantly different (Least square means, Ls mean=1.77) from the population on the other four clones. No significant difference in the number of whiteflies was found in clones 81/01635 (Ls mean 1.65), 94/0239 (Ls mean 1.61) and 92/0520 (Ls mean 1.55), while TMS 30572 supported the least number of whiteflies (Ls mean = 1.44). No significant ($P<0.05$) difference was observed in the population densities of *B. tabaci* between the two growing seasons.

The dynamics of *B. tabaci* populations during the 1996/97 and 1997/98 growing seasons were similar. Extremely low population levels, sometimes zero, were observed between the months of June/July and December (Figs. 1 and 2). Before the onset of the rains, the insect population started to build up in the last half of December. This was a period of low rainfall, relatively high temperatures and low humidity. Between February and April, when fresh leaves started to grow, an upsurge in the *B. tabaci* population was recorded. This applied to all the five clones, with the most leafy clone, TMS 91/02327, holding the highest insect number. When regression analysis was performed, strong statistical evidence was obtained for a quadratic relationship (Fig. 3) existing between the whitefly population and plant age ($F = 0.0688$, $P = 0.7958$).

Virus, vector, host and the climate

The incidence and severity of ACMV were found to vary significantly ($P<0.01$) with cassava clones and the age of the cassava plant. A significant ($P<0.01$) interaction was also observed between age and genotype of cassava plant with respect to ACMV incidence and severity. In Duncan's multiple range test, the incidence of ACMV was significantly higher in clones 81/01635 and 92/0520 than in TMS 30572 and 94/0239, while it was lowest in 91/02327. ACMV symptoms were most severe in clone 81/01635 and least severe in 91/02327 and 94/0239. During the two growing seasons, clones 94/0239 and 91/02327 remained completely symptomless except for one or two plants, which expressed only negligible symptoms.

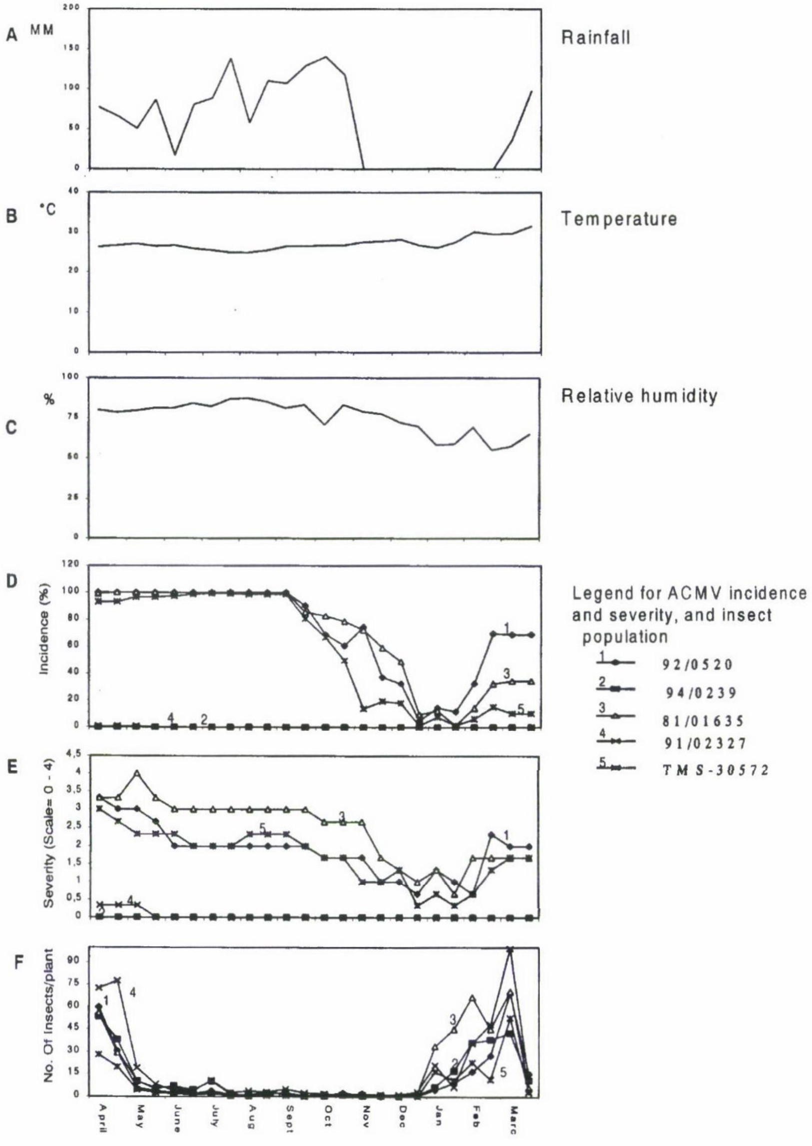

Fig. 1. Rainfall distribution (A), temperature (B) and relative humidity (C), and their effects on the dynamics of the *Bemisia tabaci* population (F), and the incidence (D) and severity (E) of African cassava mosaic virus during the 1996/97 growing season. Observations were made on 5 different clones of cassava: 94/0239, 92/0520, 81/1635, 91/02327 and TMS 30572

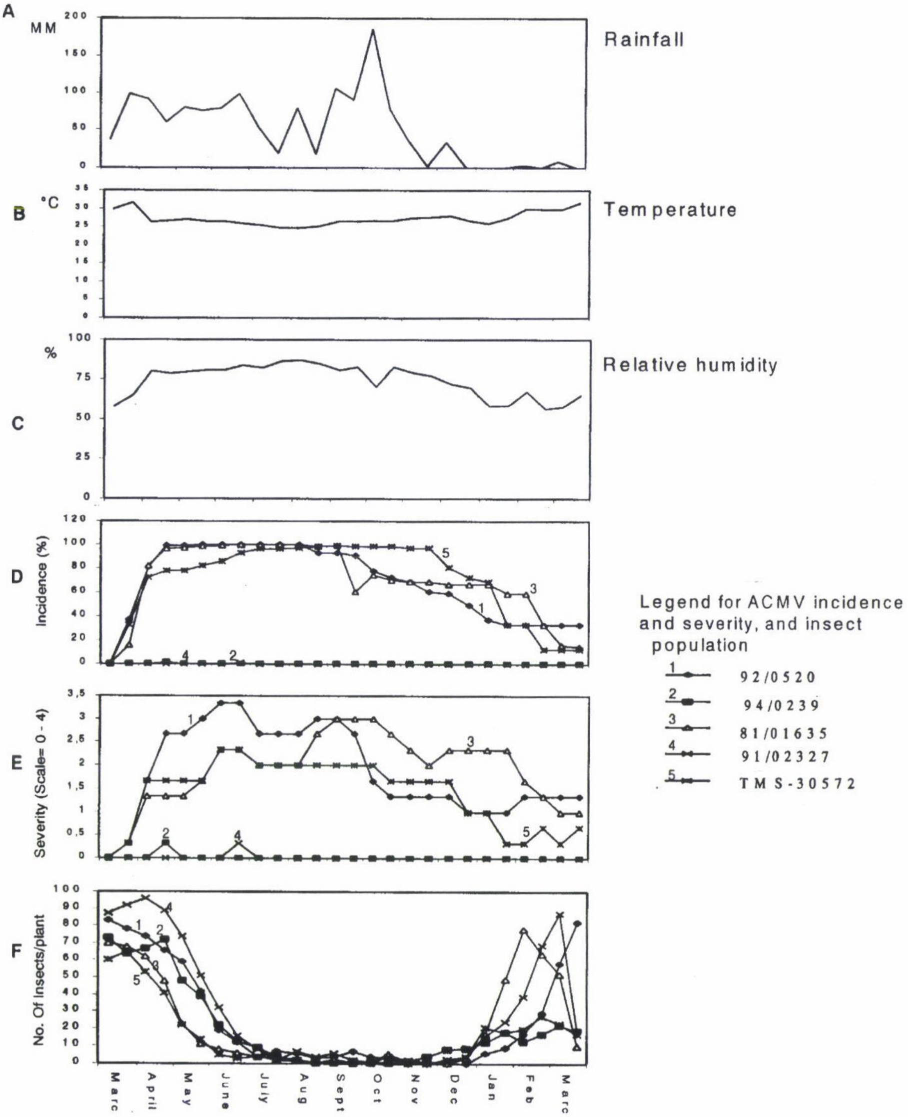

Fig. 2. Rainfall distribution (A), temperature (B) and relative humidity (C), and their effects on the dynamics of the *Bemisia tabaci* population (F), and the incidence (D) and severity (E) of African cassava mosaic virus during the 1997/98 growing season. Observations were made on 5 different clones of cassava: 94/0239, 92/0520, 81/01635, 91/02327 and TMS 30572

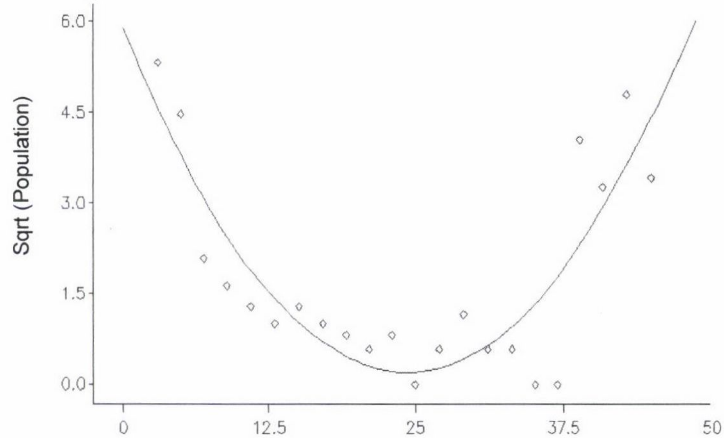

Fig. 3. Regression (quadratic) of the *Bemisia tabaci* population on the age of its host plant, cassava. The Y axis shows the number of adult *B. tabaci* (transformed) per cassava plant. The X axis reflects the week after planting the cassava

The number of infected plants in clones 81/01635, TMS 30572 and 92/0520 remained constantly high between April and September, while for TMS 30572 this period extended until November in the second growing season (Figs. 1 and 2). A gradual decline in disease incidence, starting in late September, was recorded until January of the following year. With the development of new cassava leaves, the percentage of symptomatic plants began to rise again in February. Disease severity followed a similar pattern. Clone 94/0239 was symptomless throughout the growing period, while in 91/02327 the disease severity remained mild. No correlation was observed between whitefly population density and ACMV disease incidence and severity. Rainfall, relative humidity and temperature were found to significantly ($P<0.01$) affect both the incidence and severity of ACMV, with rainfall having the greatest effect. Incidence and severity correlated positively with rainfall (0.77 and 0.71) and relative humidity (0.8 and 0.63), while a negative correlation was observed with temperature (–0.69 and –0.38).

A negative correlation between the whitefly population and the amount of rainfall (–0.266) and relative humidity (–0.516) was observed, whereas the whitefly population increased with increasing temperature. However, in general, the association of whitefly population and the three climatic parameters recorded was statistically weak ($R=0.1633$). A significantly low population of *B. tabaci* corresponded with the periods of high rainfall, during which the relative humidity ranged between 81% and 86%, with relatively low values recorded in the early part of October. The onset of rain in March stimulated fresh vegetative growth in cassava and thereby provided good conditions for whitefly population growth. In addition, disease symptoms due to ACMV became more prominent in the fresh growth of plants that had previously recovered from infection, hence explaining the increase in disease incidence (Figs. 1 and 2).

Discussion

Significant variations were observed in the population of *B. tabaci* on the five different cassava clones used in this study. Although no conscious effort has yet been made to characterize these cassava clones with regard to their suitability or acceptability as hosts for *B. tabaci*, the genotype played a major role. Differences in the hydrogen cyanide (HCN) content of cassava leaves have earlier been observed to cause variations in *B. tabaci* populations (Dengel, 1981), with varieties low in HCN carrying a higher number of whitefly. Contrarily, Cock (1973) held the view that secondary compounds such as glucosides may act as attractants or stimulants to the insects. The key factor appears to be coevolution (Bellotti, 1999); generalist feeders like whitefly, which have not coevolved with cassava, may be deterred from causing damage. The HCN content of the cassava clones used in this study was not determined, and hence there is no evidence to show the effect of this factor on the *B. tabaci* population.

No morphological feature has been found to be associated with the cyanogenic glucoside level in cassava (Rogers and Flemmings, 1973). Drought and shading have been found to increase the glucoside content of leaves (De Bruijn, 1973). This supports the argument that dry conditions significantly reduce the whitefly population (Fargette et al., 1990). Aging affects host plant suitability; high *B. tabaci* populations were observed in the five cassava clones up to the 5th week after planting. During this time, young, succulent leaves were produced, which favoured the insect. The poor nutritional quality of the leaves (Dengel, 1981; Fishpool et al., 1995) contributed, among other factors, to the low population of *B. tabaci* observed between May and December. However, clones with the ability to produce auxiliary growth provided young leaves for the insects and hence caused a rise in population. This explained the lack of correlation between the age of the five cassava clones and the population density of *B. tabaci.*

The *B. tabaci* population was not affected by the virus infection status of the five cassava varieties, and no correlation was observed between the *B. tabaci* population and the colour of the petiole. The lack of correlation between the *B. tabaci* population and ACMV incidence observed in this present study supports the findings of Fauquet et al. (1988) who saw no direct link between these two parameters. In many field and cage experiments (Robertson, 1986; Fauquet et al., 1988; Abdullahi, 2001), the total number of adult *B. tabaci* was found to have no significant influence on the incidence of ACMV. It was therefore not surprising to observe in this study that cassava clone 91/02327, with the least ACMV incidence, supported the highest whitefly population. Biochemical tests such as ELISA can be used to measure the virus infection level of the cassava clones, but this measurement was not done in the present case. Nonetheless, evidence has shown that the virus concentration in the source plant does not influence the frequency of virus transmission (McGrath and Harrison, 1995).

Moreover, no difference was found in the viral DNA content of *B. tabaci* feeding on a mildly symptomatic plant compared to that of an insect that fed on a severely infected plant (Zeidan and Czosnek, 1991). Although these findings were recorded for Tomato yellow leaf curl virus, the same argument might apply to ACMV.

There was little variation in the temperature in the study area. When temperatures were high (29–30°C) in February and March, a high whitefly population was recorded. However, high temperatures alone could not have caused the increase in the insect population, as a high population was also recorded in April and May, when the temperatures were relatively low (26°C). In many other studies (Powell and Bellows, 1992; Fishpool and Burban, 1994), however, high temperatures have been reported to favour *B. tabaci* development up to a maximum of 33°C. A negative correlation, which contrasts with the report of Dengel (1981) but agrees with Golding (1936) and Fishpool et al. (1995), was recorded between rainfall and whitefly population.

Heavy rainfall has a mechanically damaging impact (Golding, 1936) but enhances the growth of fresh leaves, thus favouring feeding activity. The differences observed in the incidence and severity of ACMV among the five cassava clones are due to genotypic factors. Clones 94/0239 and 91/02327, in which no incidence or least incidence was recorded, have been screened for four years and found to be resistant to ACMV. The resistance observed in these two clones is due not to vector repellent characteristics but rather to inherent resistance to the virus. However, Bellotti and Kawano (1980) observed a reduction in ACMV incidence when cassava varieties resistant to *B. tabaci* were planted.

Elsewhere (Gibson, 1994), TMS 30572 has been mentioned as being resistant. However, in this study and that of Ariyo et al. (2003), the incidence of ACMV in this clone was observed to be high. A recovery phenomenon (Rossel et al., 1992; Njock et al., 1996) was observed in all the infected cassava clones, with a reduction from 100% in some cases to about 10% in others. Some of the recovered plants, however, were later reinfected because of the invasion of fresh growth by the virus. The resistance displayed by clone 91/02327 and the very mild susceptibility of 94/0239 offers an encouraging control option, and they could be multiplied for subsequent release to farmers.

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EFFECTS OF ORGANIC AND INORGANIC FERTILIZATION ON WHEAT QUALITY

T. KISMÁNYOKY and I. RAGASITS

GEORGIKON FACULTY OF AGRICULTURE, UNIVERSITY OF VESZPRÉM, KESZTHELY, HUNGARY

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The influence of organic and nitrogen fertilization on the amount and quality of wheat yield was examined in Keszthely on Ramann's brown forest soil containing an average level of potassium, a low level of phosphorus and a medium level of nitrogen. The experiment involved treatments with 0–200 kg/hectare of nitrogen, 100 kg/hectare each of phosphorus (P_2O_5) and potassium (K_2O), farmyard manure, straw and green manure, together with a non-fertilized control. Nitrogen fertilization had a substantial effect on the yield (the 1.98 t/hectare yield was increased threefold by 200 kg/hectare of nitrogen). The treatments modified the quality of wheat significantly. Nitrogen fertilization together with farmyard manure increased the gluten content (to 35.8% compared to 11.35% in the control). The farinographic index increased to 77.4 (from 33.9 in the control) and the Zeleny number also increased significantly (from 10 in the control to 35.5). When low rates of nitrogen were applied overall improvement was not achieved in spite of the favourable influence of farmyard manure.

Key words: organic manure, N fertilization, wheat quality

Introduction

It is well known from Hungarian and international publications that the supply of nutrients has a decisive role in the improvement of wheat quality. Many of the publications are confined to showing the influence of artificial fertilizers, because these are capable of exerting a significant influence in the short run. However, the IOSDV (Internationale Organische Stickstoff-dauerdüngungversuche), which has been in progress for nearly two decades, enables us to keep track of the cumulative influence of manuring.

At the present level of farming the supply of nutrients to wheat has great significance. A large quantity of nutrients are given in the form of artificial fertilizers. According to Lelley (1971) artificial fertilizers are the most important agrotechnical factors for wheat. A similar opinion was expressed by Bocz (1963), who analysed the data of several countries and showed a connection between the use of artificial fertilizers and the yield of wheat.

According to Kismányoky (1999), without the use of fertilizers, at the present agrotechnical level (biological basis, machinery, plant protection) Hungarian cereal crop yields could be estimated at 2–3 t/hectare, while the rational use of manure and artificial fertilizers could increase it to 5–10 t/hectare, depending on the plant species and varieties. He also points out the great influence of plant nutrients on quality.

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According to Láng (1976) besides mineral fertilization, the nutrient supplies of the soil, manuring and a wise choice of crop sequence have a very important role as well. Győri and Győriné (1998) considered the nutrient supply to be one of the most important cultivation factors determining the quality of wheat. Kübler (1994) emphasised the effects on quality of nitrogen fertilizers, the economic efficiency of which is greatly influenced by the soil type. Jolánkai (1985) and Ruzsányi (1985) attached great importance to an adequate nutrient supply even when irrigation was possible.

Among the agrotechnical factors the nutrient supply has a decisive role in the formation of crop yield and quality (Ragasits, 1998). However, suitable plant nutrition depends on the requirements of the plant and on various environmental and agrotechnical factors. Many macro-, meso- and microelements take part in the nutrition of wheat, as in the case of other plants, but only three macroelements (N, P, K) are generally considered in fertilization practice.

Materials and methods

As part of an international cooperation a split-plot experiment with three replications was set up in Keszthely on Ramann's brown forest soil in 1983, with the crop sequence maize – winter wheat – winter barley. The plot size was 48 m^2. The wheat variety Mv 21 was used in the experiment. Results are presented for the years 2000 and 2001. The soil contained medium quantities of nitrogen and potassium and poor supplies of phosphorus (Table 1).

The treatments applied in the experiment are shown in Table 2. These were divided into three blocks. The a_1 block was treated only with artificial fertilizers, in the a_2 block the forecrop, maize, was treated with 35 t/ha manure every three years, while in the a_3 block winter barley and wheat straw and maize stalks were ploughed in with the addition of nitrogen (1 kg N/100 kg dry material). After winter barley, fodder radish (*Raphanus sativus var. oleiformis*) was applied as green manure. Quality control was carried out in accordance with the relevant Hungarian standards (ISO).

Table 1
Main soil characteristics of the experimental site

Soil properties	Soil layer, cm		
	0–30	30–60	60–90
pH (H_2O)	7.2	7.6	8.0
pH (KCl)	7.1	7.5	7.6
$CaCO_3$ %	–	5.9	27.3
Total N %	0.12	0.03	0.02
Humus %	1.7	1.4	0.8
P_2O_5 mg/kg (AL)	55	30	9
K_2O mg/kg (AL)	152	125	80

Table 2
Treatments applied in the experiments (kg/ha)

Treatments		Autumn			Farmyard	Spring N	
		N	P_2O_5	K_2O	manure	Feekes 2–3	Feekes 6
	Control	–	–	–	–	–	–
a_1	N_0	–	100	100	–	–	–
	N_1	50	100	100	–	–	–
	N_2	50	100	100	–	50	–
	N_3	50	100	100	–	50	50
	N_4	100	100	100	–	50	50
a_2	N_0	–	100	100	35 t/ha	–	–
	N_1	50	100	100	35 t/ha	–	–
	N_2	50	100	100	35 t/ha	50	–
	N_3	50	100	100	35 t/ha	50	50
	N_4	100	100	100	35 t/ha	50	50
a_3	N_0	–	100	100	–	–	–
	N_1	50	100	100	–	–	–
	N_2	50	100	100	–	50	–
	N_3	50	100	100	–	50	50
	N_4	100	100	100	–	50	50

(a_1) Mineral fertilizer only, (a_2) FYM every third year, (a_3) Straw+green manure

Results and discussion

As a result of the treatments significant differences were observed in the crop yields (Figure 1, Table 3). Manuring led to a slight increase in yield, but its effect lagged behind that of the nitrogen fertilizers. The thousand-grain mass and hectolitre weight also increased as the result of artificial nitrogen fertilizers, but higher rates resulted in a decrease in both parameters. The decrease in thousand-grain mass was moderated by manuring (a_2) and by straw+green manure (a_3) (Table 3).

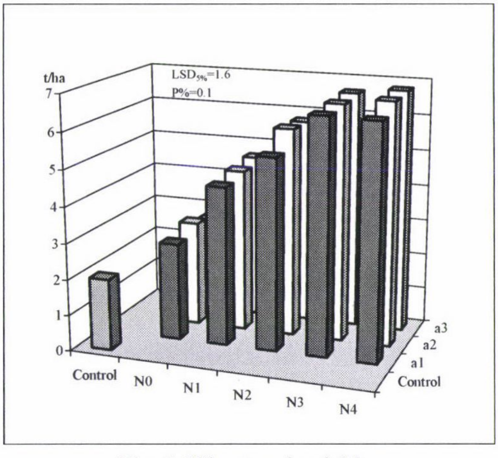

Fig. 1. Wheat grain yield

Table 3
Grain yields, thousand-grain masses and hectolitre weights measured in the experiment

	Treatments	Yield, t ha^{-1}	Thousand-grain mass, g	Hectolitre weight, kg
	Control	1.98	34.8	73.40
a_1	N_0	2.71	39.0	75.17
	N_1	4.42	41.5	76.10
	N_2	5.34	40.8	79.05
	N_3	6.52	39.0	77.85
	N_4	6.48	34.8	76.30
a_2	N_0	2.94	40.5	75.15
	N_1	4.54	40.6	76.55
	N_2	5.82	38.1	76.95
	N_3	6.58	37.0	76.30
	N_4	6.74	38.1	75.85
a_3	N_0	2.69	41.4	75.05
	N_1	4.63	41.4	76.50
	N_2	5.76	39.4	77.65
	N_3	6.64	37.7	76.10
	N_4	6.79	36.2	75.40
	$LSD_{5\%}$	1.06	7.4	–

The quality of wheat was modified remarkably by the treatments. The wet gluten content increased significantly as an effect of artificial fertilizing (Table 4). The application of manure resulted in a further increase. The best results were achieved by straw manuring at high rates of nitrogen.

There was also an improvement in the gluten class as an effect of both nitrogen fertilizing and manuring (Table 4). With the application of manure, quality classes II and I could be achieved at a lower level of nitrogen fertilization.

The farinographic index showed differences similar to those for the wet gluten content (Fig. 2). An increase in the dose of artificial fertilizers was accompanied by an increase in the index, which rose still further when manure was applied. Nitrogen fertilizer and manure changed the C1 quality category of the control treatment to A2. The change in the Zeleny number was similar to the other quality parameters. When applying higher rates of fertilizer the change was significant.

The results of the quality examinations show clearly that the quality of the control treatment and the treatment with low rates of nitrogen fertilizers did not reach the requirements of the Hungarian standard for bread wheat (MSz. 6383:1998).

As a consequence, in the soil of Keszthely, which was classified medium by Hankóczy (1930), suitable quality cannot be reached with the good quality variety Mv 21, which has a high nutrient requirement, even with manuring, if artificial nitrogen fertilizer is not used.

Table 4
Quality parameters measured in the experiment

Treatments		Gluten %	Gluten class	Gluten spreading (mm)	Farinographic index	Farinographic class	Zeleny number
	Control	11.3	III	2.3	33.9	C1	10.0
a_1	N_0	10.0	III	1.5	38.5	C1	15.0
	N_1	12.0	III	1.5	36.5	C1	17.0
	N_2	20.0	III	2.3	35.4	C1	25.0
	N_3	30.0	II	3.0	51.8	B2	30.5
	N_4	32.3	I	3.5	68.0	B1	30.0
a_2	N_0	11.8	III	1.5	38.5	C1	10.0
	N_1	23.9	II	4.0	49.2	B2	20.0
	N_2	30.6	II	4.5	57.9	B1	22.0
	N_3	34.3	I	4.5	77.4	A2	34.5
	N_4	35.8	I	3.8	74.6	A2	35.5
a_3	N_0	14.4	III	1.5	36.9	C1	18.0
	N_1	15.0	III	1.5	39.2	C1	18.5
	N_2	30.6	II	2.5	51.8	B2	28.5
	N_3	35.0	I	3.0	67.2	B1	35.5
	N_4	37.0	I	4.5	72.5	A2	32.5
	$LSD_{5\%}$	7.8	–	1.2	0.4	–	1.02

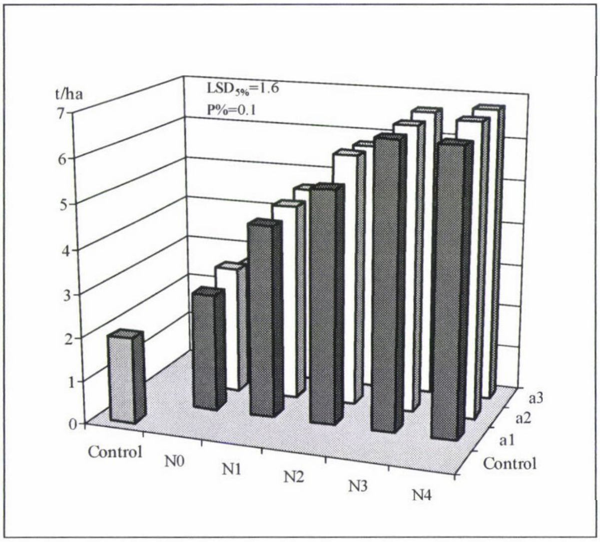

Fig. 2. Farinograph index

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COATING OF PRILLED UREA WITH ECOFRIENDLY NEEM *(Azadirachta indica* A. Juss.*)* FORMULATIONS FOR EFFICIENT NITROGEN USE IN HYBRID RICE

S. SINGH and Y. S. SHIVAY

DIVISION OF AGRONOMY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

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A field experiment was carried out during the rainy season (June–October) of 1998 at the Research Farm of the Indian Agricultural Research Institute, New Delhi, India to study the effect of coating prilled urea with eco-friendly neem (*Azadirachta indica* A. Juss.) formulations in improving the efficiency of nitrogen use in hybrid rice. The experiment was laid out in a split-plot design with three replications. Two rice cultivars, hybrid rice (NDHR-3) and Pusa Basmati-1, formed the main plots, with the levels of nitrogen (0, 60, 120 and 180 kg N ha^{-1}) and various forms of urea at 120 kg N ha^{-1} in the sub-plots. The results obtained in this study showed that the rice hybrid NDHR-3 performed significantly better than the scented variety Pusa Basmati-1 for almost all the agronomic traits tested (growth, yield attributes, grain and straw yields, nitrogen uptake and apparent N recovery)

The advantage of grain yield in hybrid NDHR-3 was nearly 16 q/ha over Pusa Basmati-1. Increasing levels of nitrogen significantly increased the number of effective tillers $hill^{-1}$, panicle length, panicle weight, grain and straw yields and nitrogen uptake, thereby revealing a significant decline in agronomic nitrogen use efficiency (NUE). Among the sources of N, Pusa Neem Golden Urea proved to be significantly superior to other sources with regards to panicle length, grain yield, N uptake, agronomic nitrogen use efficiency and apparent N recovery (%), indicating that coating urea with neem formulations not only increased the grain yield, NUE and apparent N recovery, but also helped to reduce the environmental hazards associated with the use of large amounts of urea.

Key words: hybrid rice, Pusa Basmati, nitrogen, neem formulations, coated urea, growth and yield attributes, yields, agronomic N use efficiency, apparent N recovery (%)

Introduction

Rice (*Oryza sativa* L.) is the staple food for nearly 40% of the world's population. About 90% of the world's rice is grown and eaten in Asia (De Datta, 1981), the continent which absorbs the major share of the global population increase. Fertilizer nitrogen has played a key role in increasing rice production in Asia, yet its use efficiency is only 30–40% or even less, because about 30–50% of the nitrogen applied is lost by runoff, leaching, ammonia volatilization and denitrification (Prasad, 1998a)

The use of nitrification inhibitors (NI) along with nitrogen fertilizers has been suggested as a way of increasing nitrogen use efficiency (NUE) and the two most widely tested and marketed nitrification inhibitors are 2-chloro-6-trichloromethyl-pyridine and dicyandiamide (Amberger, 1986; Prasad and

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Power 1995; Trenkel, 1997). However, poor farmers in Asian countries who cannot even afford the fertilizer can hardly pay for nitrification inhibitors. Bains et al. (1971) were the first to report increased NUE after treating urea with an ethanol extract of neem seed. Scientists at the Indian Agricultural Research Institute, New Delhi, India reported the nitrification-inhibiting properties of neem (Reddy and Prasad, 1975; Thomas and Prasad, 1983) and neem-cake coated urea (NCU) was developed and found to have higher NUE than prilled urea (Prasad and Prasad, 1983). This has been accepted by the farmers. Scientists at IARI have also experimented with neem, oil and a urea – neem oil product (10% by weight of urea) was developed and found superior to prilled urea for rice (Prasad et al,. 1998); this product is termed Pusa Neem Golden Urea (PNGU). However, urea-producing factories cannot procure sufficiently large amounts of neem oil or cake so the technology can at best be adopted at the cottage industry level. The present study was carried out to find out the efficiency of different formulations of neem-coated urea (an indigenous material with nitrification inhibitor properties), which could also promote the development of industry.

Materials and methods

Site and Soil

A field experiment was conducted at the Indian Agricultural Research Institute, New Delhi (28° 38' N latitude, 77° 11' E longitude) during the rainy season (June to October) of 1998. The soil of the experimental plot was sandy loam with pH 7.9 (soil to solution ratio 1:2.5), CEC 15.2 m.e. per 100 g soil, organic C 0.46%, total Kjeldahl N 810 ppm, 0.5N $NaHCO_3$-extractable P 0.7 ppm and 1N NH_4OH-extractable K_2O 100 ppm

Experimental design and treatments

The experiment was laid out in a split plot design with three replications. The rice varieties Pusa Basmati-1 and hybrid rice variety NDHR-3 formed the main plots, while the sub-plots consisted of the levels of nitrogen (0, 60, 120 and 180 kg N/ha), the N carriers, namely urea, Pusa Neem Golden Urea (PNGU), Karanj Emulsified Coated Urea (KEU), Pusa Neem Oil Micro-Emulsion Coated Urea (PNME), Tri-neem Coated Urea (TNU) and Neem Cake Coated Urea (NCU) prepared by coating with 2% neem cake powder by weight of urea with the help of coaltar and kerosene (1:2) at a rate of 2 ml/100 g urea and applied at 120 kg N/ha. A uniform dose of 50 kg P_2O_5 ha^{-1} was applied as single superphosphate before transplanting. Thirty-day-old seedlings were transplanted 2–3 to a hill at 20 cm × 10 cm spacing on July 26, 1998.

Field techniques

The whole quantity of nitrogenous fertilizer was applied at one time, i.e. 10 days after transplanting the rice seedlings. The rice field was kept submerged for the whole crop-growing period except the 10 days before harvesting. Otherwise the crop was grown with the usual technology. Before harvesting, the growth parameters and yield attributes of rice were recorded. The rice crop was harvested in the last week of October, 1998 and sun dried for four days in the field, after which the total biomass yield was recorded. After threshing, cleaning and drying, the grain yield was recorded. The straw yield was obtained by subtracting the grain yield from the total biomass yield. Yields were expressed in q/ha.

Laboratory chemical analysis

The plant samples were dried, ground, sieved and analysed for total nitrogen content in the plant samples at harvest using a modified Kjeldahl method (Prasad, 1998b). The N content in the straw and grain was expressed as a percentage. Nitrogen uptake by the rice crop was calculated by multiplying their respective chemical concentrations by the dry matter yield in q/ha.

The agronomic N use efficiency (kg grain kg^{-1} N applied) was calculated using the following equation:

$$NUE = \frac{\text{Grain yield}_F - \text{Grain yield}_C}{\text{Fertilizer N applied}}$$

where F = Fertilized plot; C = Control plot

The apparent N recovery (%) was determined using the following equation:

$$\text{Apparent N recovery (\%)} = \frac{N_t - N_0}{N_a}$$

where N_t = amount of nitrogen taken up from the test plot (kg ha^{-1}), N_0 = amount of nitrogen taken up from the control plot (kg ha^{-1}), N_a = amount of nitrogen added (kg ha^{-1}).

Statistical analysis of the data

All the data recorded during the experiment were subjected to computer analysis using split plot design software according to Duncan's multiple range test significant at the 95% level of probability.

Results and discussion

Growth and yield attributes

Except for 1000-grain weight (g), all the growth and yield attributes, such as plant height (cm), number of effective tillers $hill^{-1}$, panicle length (cm) and panicle weight (g), differed significantly between the varieties. Hybrid NDHR-3 produced significantly higher values of these growth and yield attributes than Pusa Basmati-1, the difference being 1.7, 33.3, 4.1 and 8.2% for plant height, number of effective tillers $hill^{-1}$, panicle length and panicle weight, respectively (Table 1). This corroborates the findings of Subramanian and Sivasubramanian (1986) and Lokaprakash et al. (1992). Nitrogen application significantly increased the number of effective tillers $hill^{-1}$, panicle length and panicle weight, but plant height and 1000-grain weight did not differ significantly. A significant increase in yield attributes with an increase in the level of fertilizer nitrogen was also reported by Kanungo and Roul (1994) and Shivay et al. (2000).

None of the growth and yield attributes except panicle length differed significantly due to the sources of nitrogen. However, a beneficial effect was observed on all yield attributes as the result of applying modified urea materials compared to prilled urea. The superior effect of modified urea fertilizers on yield attributes compared with PU confirms the findings of Prasad et al. (1999) and Shivay et al. (2000).

Table 1
Growth and yield attributes of hybrid and basmati rice as influenced by modified urea materials

Treatments	Plant height (cm)	No. of effective tillers/hill	Panicle length (cm)	Panicle weight (g)	1000-grain weight (g)
Varieties					
PB-1	88.5	5.7	24.2	1.94	19.6
NDHR-3	90.0	7.6	25.2	2.10	20.1
SEm±	0.43	0.07	0.13	0.01	0.23
CD (P=0.05)	1.30	0.23	0.40	0.04	NS
Levels of N (kg/ha)					
0	89.1	6.2	23.3	1.81	19.6
60	88.7	6.5	24.1	1.92	19.6
120	89.9	6.7	24.9	2.07	19.7
180	89.5	7.3	26.4	2.28	20.5
SEm±	0.60	0.11	0.18	0.02	0.34
CD (P=0.05)	NS	0.32	0.57	0.06	NS
Sources of N at 120 kg N/ha					
PU	89.9	6.7	24.9	2.07	19.7
PNGU	90.1	6.8	25.6	2.13	20.1
KEU	90.1	6.9	25.6	2.08	20.2
PNME	89.2	7.0	26.0	2.10	19.9
TNU	90.6	6.9	26.3	2.16	20.5
NCU	90.5	7.0	26.3	2.16	20.4
SEm±	1.01	0.09	0.17	0.04	0.37
CD (P=0.05)	NS	NS	0.51	NS	NS

PU= Prilled urea; PNGU= Pusa Neem Golden Urea; KEU= Karanj Emulsified Coated Urea; PNME= Pusa Neem Oil Microemulsion Coated Urea; TNU= Tri-neem Coated Urea; NCU= Neem Cake Coated Urea.

Grain and straw yields

All the factors, namely varieties, levels and sources of nitrogen, significantly influenced the grain yield of rice. Hybrid NDHR-3 produced significantly higher grain and straw yields than Pusa Basmati-1, the difference being 68.9 and 65.0%, respectively (Table 2). This heterosis for yield in NDHR-3 when compared to Pusa Basmati-1 was found to come from vegetative growth and also from simultaneous heterosis for a number of yield attributes, as revealed in the present study. Nitrogen application significantly increased the grain and straw yields, and the maximum grain and straw yields were recorded with the highest level of nitrogen. The application of 60, 120 and 180 kg N ha^{-1} resulted in increases of 8.7, 14.6 and 16.7%, respectively, over the control for grain yield. Data on straw yield also followed this pattern. All neem-coated materials produced higher grain and straw yields in rice than prilled urea. Among the sources of nitrogen, PNGU gave the highest grain and straw yields, significantly more than TNU, which in turn was significantly more than PNME, which in turn was significantly superior to KEU, NCU and PU (Table 2). Prasad et al. (2001) also reported that urea coated with neem formulations gave 12–13% higher rice yield than uncoated urea on the IARI Research Farm and 6–12% higher on farmers' fields.

Table 2

Yields (q/ ha), N uptake (kg/ha), agronomic N use efficiency (kg grain/kg N applied) and apparent N recovery (%) of hybrid and basmati rice as influenced by modified urea materials

Treatments	Grain yield	Straw yield	N uptake			Agronomic N use efficiency	Apparent N recovery
			Grain	Straw	Total		
Varieties							
PB-1	23.50	45.2	24.5	26.6	51.1	1.62	7.31
NDHR-3	39.70	74.6	40.7	45.4	86.1	3.53	14.2
SEm±	0.47	0.87	0.58	1.18	1.76	0.42	0.64
CD (P=0.05)	1.38	2.65	3.51	7.18	10.69	NS	3.90
Levels of N (kg/ha)							
0	28.7	54.6	26.9	28.9	55.8	–	–
60	31.2	59.4	31.3	33.3	64.6	4.16	14.6
120	32.9	61.7	35.2	39.4	73.6	3.47	14.7
180	33.5	64.2	37.1	43.2	80.3	2.66	13.6
SEm±	0.48	1.23	1.10	1.11	1.69	0.49	0.64
CD (P=0.05)	1.46	3.74	3.39	3.43	5.22	1.51	NS
Sources of N at 120 kg N/ha							
PU	32.9	61.6	33.8	36.4	70.2	3.47	12.0
PNGU	36.2	67.5	38.5	44.2	82.7	6.25	22.3
KEU	32.5	63.1	33.8	38.1	71.9	3.13	13.4
PNME	34.4	64.6	36.2	40.8	76.0	4.69	17.6
TNU	35.0	65.8	37.1	42.0	79.1	5.21	19.4
NCU	34.2	64.4	35.8	40.3	76.1	4.51	16.8
Sem±	0.82	1.60	1.06	1.76	2.49	0.69	2.08
CD (P=0.05)	2.40	NS	3.13	5.19	7.35	2.03	6.12

PU= Prilled Urea; PNGU= Pusa Neem Golden Urea; KEU= Karanj Emulsified Coated Urea; PNME= Pusa Neem oil Microemulsion Coated Urea; TNU= Tri-neem Coated Urea; NCU= Neem Cake Coated Urea, NS= non significant.

N uptake

Significant differences in N uptake with regard to grain, straw and total biomass were recorded between varieties, N levels and sources of N. Hybrid rice variety NDHR-3 had 40.7 and 45.4 kg ha^{-1} N uptake in the grain and straw, respectively, as compared to 24.5 and 26.6 kg^{-1} for Pusa Basmati-1, giving NDHR-3 68.5% more total N uptake than Pusa Basmati-1. This increase in total N uptake by the hybrid rice variety was because of higher grain and straw yield than the conventional variety. Increasing levels of N increased the nitrogen uptake significantly and each successive increment of N resulted in a significant increase in N uptake over the preceding levels of N. This was true for grain, straw and total N uptake. Thus N had a distinct role in determining the nitrogen uptake in rice. As regards the sources of nitrogen, PNGU and TNU resulted in significantly more grain, straw and total N uptake than PU, while the other sources were statistically at par.

NUE and apparent N recovery

Nitrogen use efficiency (NUE) was affected significantly by the levels and sources of nitrogen, whereas the apparent N recovery was significantly influenced by varieties, levels and sources of N (Table 2). Hybrid NDHR-3 was significantly superior to Pusa Basmati-1 in respect of apparent N recovery and also had a higher value of NUE. A significant decrease in NUE was recorded when the level of N was raised from 60 to 180 kg N ha^{-1}. The highest NUE and apparent N recovery were recorded at the lowest level of nitrogen. Among the six sources of nitrogen, PNGU resulted in significantly higher NUE and apparent N recovery than PU. In general, in respect to apparent N recovery and NUE, the different N sources exhibited the following order: PNGU > TNU > PNME > NCU > KEU > PU.

The present study suggests that to achieve higher rice yields, more N uptake by rice and higher NUE and apparent N recovery, hybrid rice should be grown at higher levels of nitrogen with the use of indigenous coating materials. The coating of urea with neem formulations not only increases the grain yield, NUE and apparent N recovery but also helps in reducing the environmental hazards associated with the use of large amounts of urea.

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EFFECT OF CONTINUOUS APPLICATION OF MANURES AND FERTILIZERS ON PRODUCTIVITY OF COTTON-SORGHUM ROTATION

D. BLAISE, J. V. SINGH, M. V. VENUGOPALAN and C. D. MAYEE

DIVISION OF CROP PRODUCTION, CENTRAL INSTITUTE FOR COTTON RESEARCH, NAGPUR, INDIA

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Field experiments were conducted from 1985 through 2001 on medium deep vertisol to determine the effect of the continuous application of manure and fertilizers on a two-year cotton-sorghum rotation. The results indicate that the response to N was greatest during the initial years, while after five to six rotation cycles the yield levels declined to the zero level in the N and NK plots. The application of P along with N prevented the decline in seed cotton and sorghum grain yields. The effect was more pronounced at the higher level. K application did not result in any yield increase. Balanced fertilizer at the higher level ($N_{90}P_{19}K_{37}$) resulted in a significant yield increase over the recommended dose ($N_{60}P_{13}K_{25}$); however, the percentage increase declined with duration, indicating a decline in factor productivity. Seed cotton yields were the highest when part of the fertilizer N was applied from an organic source (farmyard manure: FYM). Of the eight years, a significant response was observed in four years at the lower level ($N_{30}P_{13}K_{25}$ + 5 t FYM ha^{-1}) and six years at the higher level ($N_{45}P_{19}K_{37}$ + 7.5 t FYM ha^{-1}), while in sorghum a response was only observed in two years. The cotton crop should, therefore, be preferred to sorghum for the application of organic manure. In areas where no organic manure is available, $N_{60}P_{13}K_{25}$ is sufficient for cotton, while sorghum needs to be fertilized at 1.5 times the recommended dose ($N_{90}P_{19}K_{37}$).

Key words: balanced fertilizer use, *Gossypium hirsutum*, integrated nutrient management, organic manure, rotation, sorghum

Introduction

In the semi-arid tropics of central India, a two-year cotton-sorghum rotation is the predominant cropping system followed. Cotton is the major commercial fibre crop and sorghum the staple diet of millions of people in this region. However, 90% of the area is rain-dependent (5.4×10^6 ha) and this is one of the principal reasons for the low productivity of cotton and sorghum. Poor soil fertility is another cause of the low yield levels. Most often, the soils in the semi-arid areas are considered to be hungry rather than thirsty, but farmers are reluctant to apply fertilizers other than N to these crops. The risks associated with crop failures due to the aberrant rainfall pattern and inadequate pest control are major disincentives to investments in fertilizers. Most of the fertilizer trials have evaluated the response of cotton (Chand et al., 1997; Prasad and Prasad, 1998; Venugopalan and Blaise, 2001) and sorghum (Raghuwanshi and Umat, 1994) singly, and not that of the cotton-sorghum rotation system as a whole. Moreover, these studies were limited to two or three years. The response to P was inconsistent and no response was observed for K (Basu, 1992); consequently P and K have not been included in the recommendation (Tandon, 1994). Dr. R. Pundarikakshudu initiated a long-term study in 1985 with the objective of studying the effect of the continuous application of mineral fertilizers and manure, either alone or in combination, on the productivity of the cotton-sorghum rotation, the results of which are presented here.

Materials and methods

Field experiments were conducted at the Central Institute for Cotton Research (CICR), Nagpur, located at 21° 9'N, 17° 7'E, from 1985 through 2001. The site has a typical semi-arid climate with a mean annual rainfall of 1050 mm, received primarily through the southwest monsoon from June-September. The soil of the experimental site is a medium deep vertisol (*Typic Chromusterts*). Surface soil analysis at the start of the experiment from the 0–0.15 m soil layer gave values of 4.1 g organic C, 0.38 g total N, 6.1 mg $NaHCO_3$-extractable P and 253.4 mg ammonium acetate-extractable K kg^{-1} soil.

The experiment was established in 1985 and continued on the same site with the fixed layout. The fertility combinations included the recommended dose (RD), 1.5 times the recommended dose, integrated nutrient management wherein 50% of the N demand is met through organic sources (INM) and complete reliance on organic sources. The 13 fertility combinations, arranged in a randomized manner, were as follows: T1: control, T2: N_{60}, T3: $N_{60}P_{13}$, T4: $N_{60}K_{25}$, T5: $N_{60}P_{13}K_{25}$, T6: $N_{30}P_{13}K_{25}$ + 5 t farmyard manure (FYM), T7: N_{90}, T8: $N_{90}P_{19}$, T9: $N_{90}K_{37}$, T10: $N_{90}P_{19}K_{37}$, T11: $N_{45}P_{19}K_{37}$ + 7.5 t FYM, T12: 10 t FYM + $P_{13}K_{25}$, T13: 15 t FYM + $P_{19}K_{37}$. Treatments T12 and T13 were modified in 1997, with the deletion of PK application, to represent a completely organic environment, in the light of heightened interest in organically grown crops. All the treatments were repeated each year on the same plot for the entire duration of the experiment (1985–2001). The gross plot size was 4.5 × 4.8 m (21.6 m^2). Net plot size for cotton was 3.0 × 4.2 m (12.6 m^2) from 1985 through 1995 and 2.7 × 4.2 m (11.34 m^2) in 1997 and 1999. The six central rows of sorghum were harvested and the grain yield was recorded from the net plot of 11.34 m^2.

Cotton was sown at a spacing of 0.60 m between rows and 0.30 m between plants from 1985 to 1995 (cultivar SRT-1 in 1985, 1987, 1989; LRA-5166 in 1991, 1993 and 1995). In 1997 and 1999, cotton (LRK-516) was sown at a spacing of 0.45 m between rows and 0.30 m between plants. Sorghum (CSH-9) was sown at a spacing of 0.45 m between rows and 0.15 m between plants. The crop was grown using standard agronomic practices. Sowing was done at the onset of the monsoon (end of June to first week of July). Pest and disease control was carried out when it exceeded the economic threshold levels. Nitrogen, in the form of urea, was applied in three split doses except in T7 and T13. Half of the N was applied as basal fertilizer and the remainder in two equal splits, one at squaring and the other at flowering in cotton and at approximately 30 and 60 days after sowing in sorghum. In treatments T7 and T13, the basal urea dose was omitted and the entire inorganic N dose was split applied. The whole of the P, as superphosphate, and K, as muriate of potash, was applied as basal fertilizer, as was the FYM, which contained 0.6% N, 0.08% P and 0.5% K on average.

Each treatment had four replications (up to 1996). The first replication was partially damaged due to earth works in April–May of 1997, and therefore data were recorded from the three replications which were intact from 1997–98 through 2000–01. The treatments were arranged in a randomized block design. The data were statistically analysed following the procedures outlined in Gomez and Gomez (1984) and the means were separated using the least significant difference (LSD) at the 5% probability level ($p<0.05$).

Results

Seed cotton yield

Data on the effect of manuring and fertilizers on the seed cotton yield are presented in Table 1. The seed cotton yield was the lowest in the control plots. In general, it was significantly lower than in the mineral fertilized plots. After five rotation cycles, the differences between the control, N and NK fertilized plots were not significant. The increase in yield over the control due to N application amounted to 54.5–95.4% at the lower level (N_{60}) and 31.8–76.4% at the higher level (N_{90}) during the first five rotation cycles. The mean seed cotton yield was 57.5% higher than the control with 60 kg N ha^{-1}, whereas it was only 41.5% more with N_{90}. A response to P was observed from 1995–96 at the lower level ($N_{60}P_{13}$ and $N_{60}P_{13}K_{25}$ vs. N_{60}, $N_{60}K_{25}$) and from 1989–90 at the higher level ($N_{90}P_{19}$, $N_{90}P_{19}K_{37}$ vs. N_{90}, $N_{90}K_{37}$). The mean increase in yield with P application was 21.8 and 57.8% at the lower ($N_{60}P_{13}$ vs. N_{60}) and higher levels ($N_{90}P_{19}$ vs. N_{90}), respectively. After five rotation cycles, the increase in yield due to P ranged from 46.5–109% and 75.4–136%, at the lower and higher levels, respectively. The application of K (NPK vs. NP and NK vs. N) did not increase the seed cotton yield. In the balanced fertilizer plots (T5 and T10) the yield levels were generally higher at the higher levels, with significant differences in three out of the first five years. After five rotation cycles, the differences between T5 and T10 were not significant. Plots given both inorganic + organic fertilizer (INM) yielded significantly better than NPK plots in six out of eight years at the higher level ($N_{45}P_{19}K_{37}$ + 7.5 t FYM ha^{-1} vs. $N_{90}P_{19}K_{37}$) and in four years at the lower level ($N_{30}P_{13}K_{25}$ + 5 t FYM ha^{-1} vs. $N_{60}P_{13}K_{25}$). The differences between the INM treatments (T6 vs. T11) were only significant in two years. Plots with FYM alone were as good as the mineral NPK fertilizer plots except in 1985, when the yield of the FYM plots was at par with the zero level.

Table 1

Effect of manuring and fertilizers on seed cotton yield (kg ha^{-1})

Treatments	1985–86	87–88	89–90	91–92	93–94	95–96	97–98	99–00	Mean
T1 Control	678	419	432	607	537	426	416	520	504
T2 N_{60}	1315	718	844	938	852	532	422	734	794
T3 $N_{60}P_{13}$	1335	745	869	918	1068	843	882	1075	967
T4 $N_{60}K_{25}$	1191	667	775	827	852	442	565	774	762
T5 $N_{60}P_{13}K_{25}$	1210	728	911	982	1216	829	968	1121	996
T6 $N_{30}P_{13}K_{25}$+5 t FYM ha^{-1}	1385	877	1162	1144	1638	951	1136	1425	1215
T7 N_{90}	1193	580	709	800	947	413	439	622	713
T8 $N_{90}P_{19}$	1432	697	1135	1184	1563	865	1035	1091	1125
T9 $N_{90}K_{37}$	1213	511	812	623	972	420	422	645	702
T10 $N_{90}P_{19}K_{37}$	1515	859	1143	1136	1517	837	866	1224	1137
T11 $N_{45}P_{19}K_{37}$+7.5 t FYM ha^{-1}	1456	914	1324	1245	2140	1146	1067	1667	1370
T12 10 t FYM ha^{-1}	665	641	1010	1081	1342	976	1213	1231	1020
T13 15 t FYM ha^{-1}	772	793	1145	1187	1702	1080	1255	1630	1195
SE m±	82.6	78.5	55.1	63.9	89.6	69.3	52.8	92.1	63.3
LSD ($p \leq 0.05$)	237	225	158	183	257	199	154	269	179

Data pooled over the years indicated no significant differences between the INM treatments, which were at par with $N_{90}P_{19}K_{37}$ and 15 t FYM ha^{-1}. The mean increase in yield with NPK + FYM over NPK was 20.5–22%. The balanced fertilizer plots ($N_{60}P_{13}K_{25}$ vs. $N_{90}P_{19}K_{37}$) were at par with each other.

Sorghum grain yield

Data on the grain yield is presented in Table 2. The sorghum grain yield in the control plot was 1643 kg ha^{-1} at the end of the first year of the rotation, which declined to 334–410 kg ha^{-1} from the second rotation cycle, except in 1992–93, when the grain yield in the control plot was 1024 kg ha^{-1}. The grain yield in the control plots was significantly lower than in the fertilized plots in the first six years. In 1998–99 and 2000–01, the grain yield in the N alone and NK fertilized plots declined to the zero level (control). The grain yield at higher doses of N (N_{90}, $N_{90}K_{37}$) was, in general, lower than the yield at lower levels (N_{60}, $N_{60}K_{25}$). The mean increase in yield due to N application over the control was 777 and 901 kg ha^{-1} at N_{90} and N_{60}, respectively. Plots receiving N alone had yield levels at par with NP and NPK up to 1994–95, except in 1990–91, at the lower level and up to 1988–89 at the higher level. Later the yield differences widened to the level of significance. The response to P was greater at the higher levels. No direct response to K was observed. In two out of the eight rotation cycles, supplementing half the N from organic sources (T6 and T11) was found to yield significantly better than inorganic fertilizers alone (T5 and T10). Data pooled over the years indicated that maximum grain yield was obtained with $N_{45}P_{19}K_{37}$ + 7.5 t FYM ha^{-1} followed by $N_{90}P_{19}K_{37}$ and $N_{30}P_{13}K_{25}$ + 5 t FYM ha^{-1}, but the differences were not significant. Supplementing half the N with FYM (T6) was significantly better than $N_{60}P_{13}K_{25}$ (T5).

Table 2

Effect of manuring and fertilizers on grain yield (kg ha^{-1}) of sorghum

Treatments	1986–87	88–89	90–91	92–93	94–95	96–97	98–99	00–01	Mean
T1 Control	1643	370	334	1024	380	410	220	336	589
T2 N_{60}	3614	1891	902	2243	1099	1374	432	367	1490
T3 $N_{60}P_{13}$	3126	1589	1590	2843	1424	2186	1267	1073	1887
T4 $N_{60}K_{25}$	3725	1614	1062	2230	1027	1167	431	457	1464
T5 $N_{60}P_{13}K_{25}$	3311	1753	1382	2630	1121	2071	1191	1277	1842
T6 $N_{30}P_{13}K_{25}$+5 t FYM ha^{-1}	3913	1928	1771	3228	1598	2918	1675	1771	2350
T7 N_{90}	3229	1853	754	1975	1003	1481	271	361	1366
T8 $N_{90}P_{19}$	3891	2124	1759	3586	1854	2592	1870	1549	2403
T9 $N_{90}K_{37}$	3406	1731	595	2166	1075	1270	458	297	1375
T10 $N_{90}P_{19}K_{37}$	3863	2224	2056	3335	1674	2533	1890	1781	2419
T11 $N_{45}P_{19}K_{37}$+7.5 t FYM ha^{-1}	3776	2009	2523	3904	2245	3000	2143	1734	2667
T12 10 t FYM ha^{-1}	2024	2694	996	2474	1521	1934	2236	2050	1991
T13 15 t FYM ha^{-1}	2128	3746	1262	2949	1700	2417	1581	2296	2260
SE m±	283.1	148.7	162.6	228.6	116.7	145.9	174.7	195.2	167.0
LSD ($p \leq 0.05$)	812	427	467	656	335	419	510	569	469

Among the balanced fertilizer treatments (T5 and T10), the application of 1.5 times the recommended dose (T10) was significantly better than the recommended dose (T5) in six out of the eight years. In 2000–01, the differences were not significant. In general, FYM alone led to yield levels at par with mineral fertilizers (NPK) alone, except in 1986–87 and 1988–89. In 1986–87, the FYM plots had yields at par with the control and significantly lower than the fertilizer plots. This trend was similar to that observed in cotton. In 1988–89, the FYM plots had the highest yield levels, which were significantly better than in the inorganic fertilizer treatments.

Discussion

The yield increases achieved through the application of fertilizer N ranged from 31.8–95.4% in cotton and 92.8–411% in sorghum during the first five rotation cycles. This indicates that N is the most limiting nutrient. The soils are deficient in N and the response to N is very distinct. It is also the main reason why farmers prefer fertilizing their crops with urea fertilizer. Earlier studies (Chand et al., 1997; Mathur, 1997) also showed the importance of N for achieving high yield levels. An interesting finding in the present study is that yield levels tended to decline when N alone was applied. The magnitude of this decline was greater at the higher levels. The continuous use of N alone cannot be relied upon to produce high yield levels. When the supply of N, the major limiting nutrient, is adequate, other nutrients in short supply affect the yield. In an 18-year study in Upper Volta, Pichot et al. (1981) reported an increase in sorghum grain yield with mineral fertilizers in the initial years. K deficiency and Al toxicity, in the later years, were found to be yield-limiting factors. In the present study, it was found that P is the second most important yield-limiting factor. A response to P was not seen in many earlier trials conducted for two to three years (Chand et al., 1997; Basu, 1992). The present findings indicate that the response to P was visible after two rotation cycles (four years) at the higher levels. The soil P is too low to avoid fertilizer P application. No direct effects of K were observed, possibly due to the high exchangeable K reserves. The mean seed cotton yield was not significantly different between the balanced fertilizer treatments (T5 and T10), whereas the sorghum grain yield was significantly better at $N_{90}P_{19}K_{37}$ than $N_{60}P_{13}K_{25}$. Sorghum is a short duration crop and the high nutrient demand within a short duration period may result in a response to higher doses. Another important finding emanating from this study is that the yield level at $N_{90}P_{19}K_{37}$ tended to be almost equal to that achieved with $N_{60}P_{13}K_{25}$ in later years. This could be attributed to the decline in nutrient use efficiency observed in earlier long-term studies in the rice-wheat system (Yadav, 1998). INM treatments (T6 and T11) were better than the balanced fertilizer treatments (T5 and T10). These results are in line with studies on cotton (Mathur, 1997) and sorghum (Gangwar et al., 1992; Pichot et al., 1981). In the initial years, treatments with organic manure alone (T12 and T13) had relatively lower yield levels. The effect of organic manure is cumulative; therefore, longer duration may be required for the effect to be noticed. Secondly, organic manure releases nutrients slowly and could possibly have limited the nutrient supply. Fries and Aita (1990) observed the N absorption of sorghum plants to be highest with mineral N and lowest with cattle manure. Obviously, these limitations may have been overcome in the INM treatments (T5 and T11), resulting in high yield levels. Relying solely on organic sources of nutrients would mean the application of at least 10 t FYM ha^{-1} to equal the yield levels obtained with $N_{60}P_{13}K_{25}$. Such large amounts of farmyard manure may not be available to meet the needs of all the cultivated land. The use of chemical fertilizers along with organic manure is probably the best option to maintain high productivity levels and keep food and fibre production ahead of the increasing population (Prasad and Power, 1997). Significantly, higher seed cotton yields were achieved in four and six of the eight years at the lower and higher levels, respectively, while in sorghum a response was seen in only two years. The peak nutrient demand in cotton occurs later during crop growth, when the N supplies typically diminish and root activity is less (Gerik et al., 1998), while in sorghum it is between 50–75 days. Most of the N in manure is in the organic form. The N-containing organic compounds in FYM are resistant to decomposition and only one-third of the N is easily released (Cooke, 1975), which does not provide an adequate amount for uptake. This slow release may coincide with the peak nutrient demand of cotton, thus explaining the better response to INM in cotton. Khiani and More (1984) reported a two-fold increase in seed cotton yield with the application of FYM, whereas the sorghum grain yield increased by hardly 28% with organic amendment.

It is therefore suggested that in areas where organic manure is in short supply, the cotton crop should be preferred over sorghum for the application of organic manure. If organic manure is not available, the farmers are advised to apply phosphate along with the nitrogenous fertilizers to achieve potential yields. The application of $N_{60}P_{13}K_{25}$ would be sufficient for cotton and 1.5 times the recommended dose would be beneficial for sorghum. Though a response to potassium was not seen, it is better to adopt a balanced fertilizer schedule (NPK application), wherein K is applied as a prophylactic measure.

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PRELIMINARY NOTES REGARDING EARLY 10TH CENTURY CEREAL PRODUCTION BY THE FIRST HUNGARIAN SETTLERS

F. GYULAI

INSTITUTE FOR AGROBOTANY, TÁPIÓSZELE, HUNGARY

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Archaeobotany, the study of plant macrofossils (seeds and fruits) obtained from archaeological excavations, becomes particularly important when there is very little or no archaeological, written or iconographical material available about the cultivation of the plants found. This is particularly the case in relation to the early Hungarian settlers.

The most significant event of the 10th century in the Carpathian Basin was the Hungarian conquest, yet this is the most fiercely debated period of Hungarian history, and the subject, in some cases, of extreme views. The information available on the way of life of the early Hungarians is very sparse, especially as regards farming and crop production skills.

The conquering Hungarians were "semi-nomadic". This may equally include mobile pastoralism and a limited extent of tillage and plant cultivation. Other archaeobotanical evidence suggests that the early Hungarians were not nomadic. There are very few seed remains directly relevant to the period of the Hungarian Conquest: the leading strata of early Hungarian society probably practised mobile pastoralism of a fundamentally Turkish character. It can be presumed that plant cultivation was the occupation assigned to common people who pursued a more sedentary way of life. It was probably these people whose plant remains were found in Lébény-Billedomb (near Győr) in 1993 and are presented in this paper. This is the first evidence of plant cultivation by the early Hungarians. The finds from the 10th century settlement are rich in cereal species such as common wheat, barley, rye and millet.

Key words: archaeobotany, plant macrofossils, cereals, early Middle Ages, Hungarian Conquest

Introduction

The general image of the conquering Hungarians has been considerably modified under the influence of research results accumulated during recent decades. Even today, however, we are still haunted by the myth that the conquering Hungarians led a "Turkish-style", equestrian, pastoral, nomadic way of life, while the duty of land cultivation, alien to this culture, was delegated to the conquered.

However, linguistic evidence, the analysis of Byzantine and Arabic written sources, as well as the modern excavations of settlements and cemeteries dated to the period of the Hungarian Conquest have gradually modified our general view on the life the early Hungarians must have led.

The Hungarians, who were originally fishers and hunters, became stock-farmers, and to some extent crop producers, during the time spent in the area of

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Levedia during the 6th to 9th centuries. Preceding the period of the Hungarian Conquest, Hungarians lived in an area where for at least three centuries they were exposed to the cultural traditions of the Saltovo-Mayack culture. This culture area was bordered by the upper reaches of the River Don to the north, the Caspian Sea and the River Volga to the east and the Crimea and Kuban to the south. Adjacent to this region to the west was the huge plain defined by the River Donyec and the Azov Sea. The Saltovo-Mayack culture cannot be associated with a single ethnic group but should rather be seen as a cultural-historical trend. Hungarians in this area lived in an economic and political alliance with the Khazar Khaganate for a long time. The Bulgarian-Turkish peoples of the Khazar Khaganate exerted a strong influence on the culture of the ancient Hungarians. It was during this time that Turkish loan-words relevant to farming entered the Hungarian language. They complemented and sometimes replaced words relevant to farming in the original Finno-Ugric vocabulary of the Hungarians. Balassa (1973) believes that nomadic migration must have been only a transient feature generated by external pressure, such as moving to Levedia and later on into Etelköz. The abandonment of the migrating lifestyle is associated with the abundance of grazing land.

The agriculture of the ethnically rather heterogeneous group of conquering Hungarians could be characterized in a single term, "semi-nomadic". This description can accommodate not only mobile animal keeping but a limited extent of land cultivation and agriculture as well.

During the period of the Hungarian Conquest approximately 1/8 of historically defined Hungary was either temporarily or permanently covered by water (Györffy and Zólyomi, 1994). In addition, the climate was favourable for agriculture, since the "small climatic optimum" between approximately 800 and 1200 was probably the warmest period over the last 2000 years. Although the climate began to grow increasingly humid around the year 1000, this trend did not become pronounced until the 13th century (Rácz, 1993).

It is very useful to compare the climate, soil type and natural vegetation of the forested steppe zone in Levedia with those in the Great Plain areas first invaded by the conquerors. A striking similarity is found (Hortobágyi and Simon, 1981). Forested steppes constitute an intermediary stage between flat grasslands and forests, where steppe and forest interchange in a mosaic pattern. The forests, rich in steppe elements, take the character of parklands. In the beginning, the early Hungarians occupied those areas of the Carpathian Basin which were most similar to the areas they had formerly inhabited. These included the sandy plains in the Nyírség region and between the Danube and Tisza Rivers. This territory corresponds to the westernmost corner of their original natural environment, the forested steppe region. In other words, the leaders of the conquering Hungarians settled in the Nyírség region, the Mezőföld Plain and the sandy quarters of the Small Hungarian Plain because these were most similar to their ancient homeland (the transitional zone between the steppe and parkland steppe). Common people, on the other hand, preferred the silty-loessy, often forested floodplain areas on the left bank of the River Tisza (eastern Hungary) and areas in Transdanubia (western Hungary), since in addition to animal keeping, land cultivation could also be successfully practised there.

According to Zólyomi (1980), the pollen samples available for analysis from the period of the Hungarian Conquest in the Lake Balaton region contained increasing proportions of grains and seeds from ploughland weeds, a phenomenon that seems to be closely correlated with an increase in the number of settlements.

The plant cultivation between the period of the Hungarian Conquest and the 12th–13th century appears to have followed a straight, continuous trend, a development with no setbacks. Cereal finds from the excavations of 12th–13th century settlements on the Great Hungarian Plain start to display species compositions similar to those of Transdanubian assemblages (Hartyányi et al., 1967/68), indicating that the inhabitants of the Great Hungarian Plain had also become sedentary by the 12th–13th century. The changes in seed materials marked a qualitative leap in plant cultivation. The cultivation of common wheat and rye, cereals with high nutritional value, became widespread and commonplace. Millet assumed only secondary importance, although it remained in cultivation in Europe as a basic ingredient of porridge until modern times.

Although the agriculture of this period must have been strongly influenced by the plant cultivation skills of peoples who inhabited the Carpathian Basin prior to the period of the Hungarian Conquest (Moravians and Franks), such an effect remains invisible. The integration of Late Avar agriculture into this body of knowledge should also be reckoned with. This information, similarly to the peoples who transmitted it to the Hungarians, must have been rapidly turned into a homogeneous unit as a feudal state was established. This means that centralized royal power, the emergence of a latifundium system, the adoption of Christianity as a state religion and the spread of literacy ensured that both know-how imported from abroad and dynamic developments in agricultural equipment spread even to the most remote parts of the country.

Materials and methods

Archaeobotany is a discipline concerned with the evaluation of seeds and fruit remains brought to light during the course of archaeological excavations. Important research areas in archaeobotany include the identification of remains from cultivated plants, the investigation of the process by which wild species became cultivated plants, and the distributions of plant cultivation and agriculture. Diasporae buried in the soil are also preserved differentially for a variety of reasons. In features connected with human habitation (refuse pits and layers, cess-pools), the evidence of ancient crop cultivation can mostly be found. These include grain with the associated remains of weeds.

The importance of archaeobotanical studies of plant macrofossilia (seeds and fruits) becomes especially significant when no or minimal archaeological, written or iconographic evidence is available for the purposes of research. Plant cultivation by the early Hungarians is such a topic.

Only a few seed remains indicative of plant cultivation are available from the period of the Hungarian Conquest (Hartyányi et al., 1967/68). This can be explained in part by the fact that it is predominantly cemeteries that are known from this period, and these are not the most typical features in which plant remains may be found. One of the few exceptions is represented by the grave of a Hungarian of high social status in Zemplén which lies beyond the Hungarian border. This burial site contained the grain of millet (*Panicum miliaceum*). Plant remains from the migration period found on the Great Hungarian Plain and in other parts of Eastern Europe show that the most important cereal cultivated by nomadic and semi-nomadic peoples was millet, whose cultivation requires relatively little attention. Consequently, millet meal, or porridge, must have been among the most important foods of these peoples (Wasylikowa et al., 1991).

To date, the only archaeobotanical assemblage connected to a settlement once inhabited by early Hungarians comes from Lébény-Billedomb (Fig. 1), a site near the city of Győr on the Small Hungarian Plain in Western Hungary (excavations directed by M. Takács in 1993). Lébény-Billedomb may be considered as the very earliest archaeobotanical material from the period of the Hungarian Conquest (beginning of the 10th century). The entire site was systematically sampled for plant remains. During the course of fieldwork in 1993, three soil samples (each 20 kg) were gathered and flotated from settlement features associated with the early Hungarians. The evaluation of this material, therefore, offers information of vital importance concerning the agricultural practices characteristic of the period. Since information on plant cultivation in this period was previously typified by unsubstantiated hypotheses and often contradictory opinions, the compositions of four archaeobotanical samples that represent the earliest period of the settlement are of outstanding significance. Considering the unusual importance of this find, results from the recently completed analysis are presented here (Gyulai unpublished).

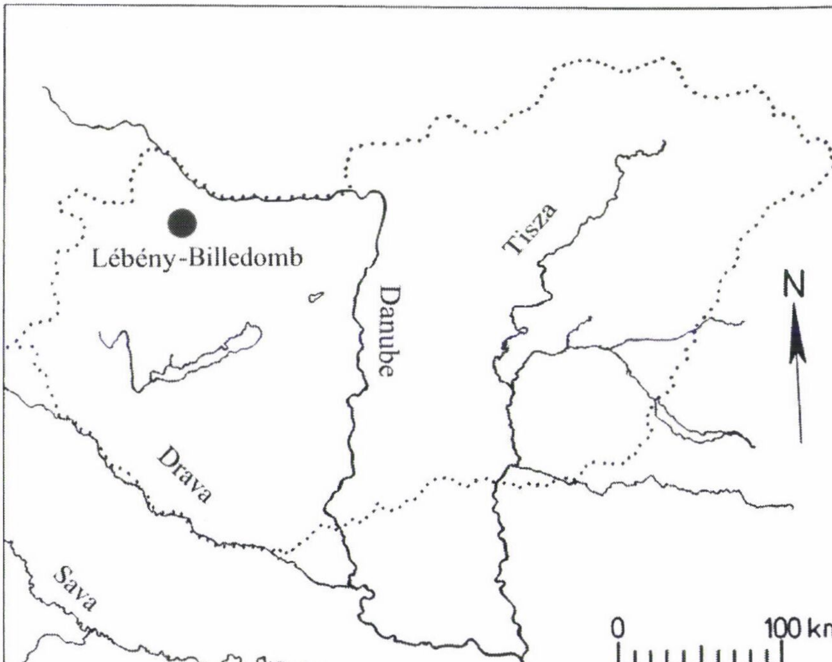

Fig. 1. Sitemap of the excavation

Results and discussion

The state of preservation of carbonized seeds in Lébény-Billedomb is good, each being coeval with the culture-bearing layer in which it was found. The material is taxonomically rich even in comparison with earlier periods: the approximately 200 seeds recovered originate from 30 plant species (Table 1).

Table 1

Plant remains from Lébény-Billedomb (beginning of the 10^{th} century)

Latin name	English name	Remains	Condition	No.
Ajuga reptans L.	carpet bugle	seed	carb.*	2
Brassica campestris L.	wild turnip	seed	carb.	1
Bupleurum rotundifolium L.	thoroughwax	achenes	carb.	1
Cerealia	cereals	grain fragment	carb.	32
Cerinthe minor L.	lesser honeywort	seed	carb.	4
Chenopodium album agg.	goose foot	seed	carb.	10
Chenopodium hybridum L.	maple-leaved goosefoot	seed	carb.	23
Chenopodium spec.	goosefoot	seed fragment	carb.	2
Euphorbia cyparissias L.	cypress spurge	seed	carb.	2
Fallopia convolvulus (L.) A. Löve	black-bindweed	seed	carb.	3
Hordeum vulgare L. subsp. *distichum* Zoh.	two-rowed barley	hulled grain	carb.	2
Hordeum vulgare L. subsp. *hexastichum* Zoh.	six-rowed barley	hulled grain	carb.	19
Hordeum vulgare L.	barley	hulled grain	carb.	15
Hordeum vulgare L. var. *nudum*	naked barley	naked grain	carb.	1
Lens culinaris Medic. subsp. *macrosperma* Bar.	large-seeded lentil	seed	carb.	1
Malus silvestris agg.	crab apple	seed	calc.	1
Melilotus albus Desr.	white melilot	seed	carb.	8
Muscari comosum (L.) Mill.	tassel-hyacinth	seed	carb.	1
Panicum miliaceum L.	common millet	naked grain	carb.	2
Poa spec.	meadow-grass	naked grain	carb.	1
Poaceae	grasses	hulled grain	carb.	1
Poaceae	grasses	node fragment	carb.	1
Polygonum aviculare agg.	knotgrass	seed	carb.	12
Polygonum persicaria L.	redshank	seed	carb.	3
Potentilla reptans L.	creeping cinquefoil	seed	carb.	1
Ranunculus repens L.	creeping buttercup	seed	carb.	3
Reseda lutea L.	yellow mignonette	seed	carb.	2
Sambucus ebulus L.	danewort	seed	carb.	5
Schoenoplectus lacustris (L.) Palla	common bulrush	seed	carb.	2
Secale cereale L.	rye	naked grain	carb.	4
Setaria viridis (L.) PB./*verticillata* (L.) R. et Sch.	green/rough bristle-grass	naked grain	carb.	2
Sinapis arvensis L.	charlock	seed	carb.	3
Stachys arvensis L.	field stitchwort	seed	carb.	2
Teucrium chamaedrys L.	wood germander	seed	carb.	1
Triticum aestivum L. subsp. *vulgare* (Vill.) MacKey	common wheat	naked grain	carb.	11
Indet.		seed fragment	carb.	16
Millet meal or porridge		fragment	carb.	5

*carb.: carbonized; calc.: calcinated

Forms of chaffed wheat, so characteristic of prehistoric sites, were not found at all. It seems that improved forms of naked common wheat (*Triticum aestivum* ssp. *vulgare*) were cultivated at this site. In addition to grain from six-row barley (*Hordeum vulgare* ssp. *hexastichum*), remains of the two-row barley (*Hordeum vulgare* ssp. *distichum*) and naked varieties of this species were also identified. Both millet (*Panicum miliaceum*) and rye (*Secale cereale*) also occur. This latter may have been grown by itself, but the combined cultivation of wheat and rye may also have been practised. It is also possible that millet was sown as a second crop.

Sub-dividing the macrobotanical finds into anthropogenic categories, a picture of varied agricultural activity emerges. The inhabitants of this settlement did not specialize in a single or only a few crops, but cultivated a broad range of cereals that included all the important species (Fig. 2). It seems likely that six-row barley, which made up the greatest number of individual finds in the material, was grown as animal fodder. Millet, common wheat and rye, on the other hand, must indisputably have served as human food.

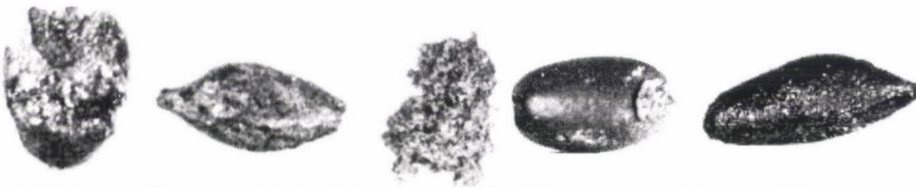

Fig. 2. The most important crop remains

As a general observation, it is worth mentioning that a high level of cereal cultivation usually coincides with the cultivation of vegetables. This phenomenon is manifested at this site as well: in addition to cereal grain, peas (*Pisum sativum*) were also identified.

Weed remains also confirm the cultivation of crops. The majority of species in this group (7) are spring cereal weeds or characteristic species of garden weed associations (*Polygono-Chenopodietalia*). A smaller group (4 species) represent typical associations of winter cereals or cereals in general (*Secalietea*).

Conclusions

Botanical finds from the Carpathian Basin and the surrounding countries indicate that during the migration period, including the time of the Hungarian Conquest, Roman agriculture was replaced by a much more modest crop production system. Although all the crops grown in former ages are still found, they are very modest in extent and the grains are smaller. The leading crop is common millet, a characteristic type of corn for quickly moving nomadic people (Wasylikowa et al., 1991). Pollen diagrams (Zólyomi 1971, 1980; Zólyomi and Précsényi, 1985) confirm these findings.

According to research scientists dealing with the history of crop production in Hungary, the most important cereals in the migration period, the age of the conquest and the time of the Árpád dynasty were common millet and barley (Rapaics, 1934; Gaál, 1978). The archeobotanical research presented here has modified this concept.

The plant remains from Lébény-Billedomb (beginning of the 10^{th} century) are the first evidence for crop cultivation by the early Hungarians. The finds of millet, two- and six-row barley, common wheat and rye prove that they must have been familiar with the technique of ploughing.

Although the bones of sheep and cattle, characteristic of a mobile lifestyle, dominate among the animal remains (Vörös, 2000), this phenomenon does not contradict the possibility that early Hungarians arrived in the Carpathian Basin with considerable agricultural expertise that included plant cultivation.

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BEHAVIOUR OF COWS AT THE FEEDING TROUGH AND WHEN ENTERING THE MILKING PARLOUR

T. GERE and G. HAMAR

DEPARTMENT OF ANIMAL BREEDING, COLLEGE OF ECONOMICS AND AGRICULTURE, SZENT ISTVÁN UNIVERSITY, GYÖNGYÖS, HUNGARY

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Observations were made during 2 × 3 days on the behaviour of 29 Holstein-Friesian cows when entering the milking parlour and at the feeding trough. The cows were identified by an electric apparatus on their necks, and by transmitters at the feeding trough in the milking parlour. Milking was done using Alfa-Laval milking apparatus with 2 × 4 milking stalls and an automatic cup-removing gadget. Rank correlation coefficients were calculated between the entrance order and the most important production characteristics.

There was no correlation between the entrance order to the milking parlour and the dominance order at the feeding trough. The younger cows were dominant when entering the milking parlour, and the older, heavier cows at the feeding trough.

Key words: cow, behaviour, dominance

Introduction

Cows kept in herds on large commercial dairy farms can often be seen in different rank positions at the feeding and drinking places, than in resting places or in the corridors of the milking stalls. The places of the cows are usually determined by the previously established hierarchy in the group. However, these facilities and production zones do not always provide suitable surroundings or effects for the cows, so it is important to know whether the cows take up these positions according to their ranking order, or whether some other factor regulates their behaviour.

No relationships have yet been found between the entrance order to the milking parlour and the other most important traits (milk production, body weight, age), but it has been observed that if the cows are crammed into a small space, for example when waiting to enter the milking parlour, the usual social ranking does not prevail.

The provision of a broader waiting place or smaller groups helped to establish a stable social hierarchy, but fluctuation in the milking time and changes in the cows' companions had a negative effect on milk production. A stable rank order at milking and a relatively small group size in the milking parlour are therefore very important. In the case of bigger groups (80–100 cows), more than 50% of the cows have different companions in the milking stall, and more than 96% of cows are milked at different times (Czakó, 1978).

Not all cows enter the milking stalls of their own free will. On the basis of how they entered the milking stall, Porzig (1969) divided cows into three groups. The first group pushes forward (Vordränger), the second group hesitates

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(Mittelgruppe), while the third lags behind (Zurückbleiber). Cows in the first group generally enter the milking stall willingly. Cows in the second group hesitate before entering, but finally enter alone in most cases, though some have to be driven into the stall. In the remaining group none of the cows enter the milking stalls of their own accord.

The order of entering the milking parlour is inherited. Macha (1981) found heritability values (h^2) of between 0.21–0.27 and 0.62–0.81 for this trait.

The social order of entrance to the milking stall remains stable if the cows are not interfered with. Some cows always choose the same side of the parlour, and some always stand at the same place within the milking stall. If these cows are driven out of their accustomed place, they will react by milk retaining, i.e. a reduction in milk production (Porzig, 1969).

The aim of the present research was to observe the order in which the cows entered the milking parlour, the order in which they occupied their places in the milking stalls, and the effect of the social ranking on the entrance sequence to the milking stalls. Correlation coefficients were calculated between these parameters and the previous and current milk production, the body weight, and the stage of lactation. Thus, for a specific keeping, feeding and milking technology, the relationship between the dominance order at the feeding trough and the entrance order in the milking parlour was tested.

Materials and methods

The observations were performed on 29 Holstein-Friesian cows during 2 × 3 days in January and February 1999 in a cow house at Szent István University (formerly: University of Agricultural Sciences, Gödöllő). The cow house had 120 stalls, with a resting place. The cows were not bound, so they could move freely in the cow house. There were two covered feeding troughs on the longer side of the cow house, where hay and other mass fodder was distributed from the passage in front of the troughs. Grain fodder was available from the automatic feeding apparatus, programmed according to the milk production of the cows. At the end of the building was the milking parlour, where the cows were milked using an Alfa-Laval milking apparatus, with 2 × 4 milking places and an automatic cup-removing gadget.

The farm was equipped with computers working on an automatic direction system, providing a knowledge of milk production, the identification of the cows at the automatic feeding cylinder, in the milking parlour and when entering the parlour, and the collection and treatment of milk production and breeding data.

The observations were made easier by a fast, exact identifying apparatus. This apparatus was placed on the necks of the cows and provided cow identification by a transmitter during walking and milking. The most important data from the management point of view (feed consumption and production) were thus available.

The social order was observed at food distribution and in the waiting place of the milking parlour. The order in which the cows occupied the milking stalls was recorded with the help of the identifying system.

To determine the dominance or subordinacy, the method of Czakó (1978) was used. This method is able to evaluate the interactive behaviour of pairs when meeting at the feeding trough (threat, attack, subordination, retirement, etc.). The rank order was calculated within the group.

Rank correlations were calculated between the dominant or subordinate positions of the cows at the feeding trough and the occupation of the milking stalls, according to Sváb (1973). Other data (daily milk production, body weight, age, milk production in the present and previous lactation) were gathered from the farm records.

Results

Order of entrance to the milking parlour

There was a relatively high (r = 0.7) correlation between the entrance order to the milking parlour over a six-day period. The entrance order therefore remained relatively stable during the observation time, but a five-stalls fluctuation was observed in the occupation of stalls by the same cow.

Relationship between dominance at the feeding trough and entrance to the milking parlour

This relationship was estimated by rank correlation. The rank position and the place of the cow in the estimated group was determined on the basis of the difference between the marks awarded for dominance and subordinacy.

The relationship between dominance values (marks) at the trough and at the waiting place was also determined. This calculation resulted in a weak, positive, r = 0.11 rank correlation. The values of dominance are illustrated in Figures 1 and 2.

A comparison of Figures 1 and 2 indicates that the dominance relations were clearly established at the feeding trough. This means that at feeding the dominance subordinacy relationship was more prevalent, but when entering the milking parlour no definite rank order could be observed among the cows. This explains the very weak connection between the two rank orders.

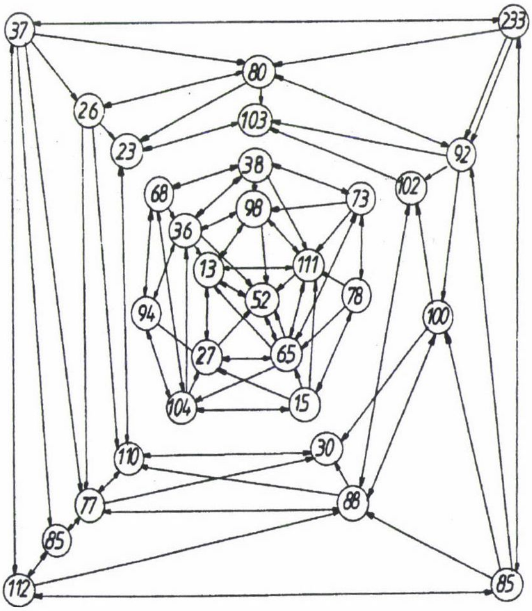

Fig 1. Dominance relations at the feeding trough

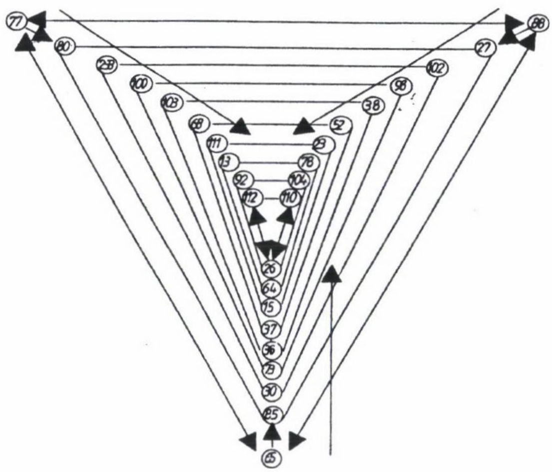

Fig. 2. Dominance relations at the entrance to the milking parlour

Relation between the rank order at the waiting place and on entry to the milking parlour

The values of entrance to the milking parlour were calculated according to the previously described method. The values showed a relatively good agreement between the position at the trough and the position in the waiting place. This was supported by the rank correlation coefficients between the observed sequences at the two places. Cows in the first half of the group tended to keep this position in the waiting place, too. A loose, positive rank correlation coefficient ($r = 0.39$) was found between the sequence of entrance to the waiting place and the sequence occupying of stalls in the milking parlour.

Connection between the order of entering the milking parlour and major economic traits

Correlation coefficients were calculated between the order of entering the milking parlour and the following traits:

– daily milk production (kg), average milk production over the six days of testing,

– body weight (kg), estimated for lack of suitable scales,

– age, taken from farm records,

– milk production during the previous lactation (kg) taking one lactation period as 305 days,

– cumulative milk production during the present lactation (kg),

– lactation period (days), from calving to the date of observation.

The cows were ranked on the basis of the above traits, in diminishing order in the case of daily milk production, body weight, age, and milk production during the previous and present lactation, while an increasing order was used to rank the lactation period, the cow with the shortest lactation period being ranked first.

The rank correlation coefficients between the order of entering the milking parlour and the observed traits can be seen in Table 1.

The data in Table 1 did not show any definite relationship between the order of entering the milking stall and the daily production. Similarly rank correlation failed to show a connection between milk production in the current lactation and the order of entering the milking parlour. There was a weak rank correlation between the previous lactation and the entrance order, with the most productive cows coming lower in the rank order. No connection was found between the entrance order and the duration of the present lactation.

A negative relationship was found between entrance to the milking parlour and both body weight and age. The older, heavier cows allowed the younger, lighter cows to precede them.

The data showed that, when entering the milking parlour, lighter younger cows that had been less productive in the previous lactation were highest in the rank order.

Table1

Rank correlation between the order of entering the milking parlour and production traits

Traits	r
Age	–0.18
Estimated body weight	–0.36
Production during the previous lactation	–0.28
Daily milk production	–0.09
Production during the present lactation	–0.06
Lactation period	+0.08

Conclusions

The order of entering the milking parlour was relatively constant on the observation days, as proved by the high ($r = 0.7$) rank correlation coefficient.

The social rank order was stable within groups of cows at the feeding trough: there was a clearly established dominance-subordinacy relationship between the cows in the group (Fig. 1). At the feeding trough the bigger, heavier, older cows had the advantage.

There was no hierarchy within the group in the order of entering the waiting place (Fig. 2). Here the younger, lighter cows tended to head the rank order.

There was no significant correlation between the rank orders observed when entering the waiting place and during feeding.

It is suggested that feeding has a positive effect on the animals, so heavier body weight and greater age prevail at the feeding trough.

The milking parlour and milking have a negative effect on the cows, so the older, heavier cows allow the younger, lighter cows to take precedence.

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Review

DEVELOPMENT OF CROP PRODUCTION TECHNOLOGIES FOR MULTIFUNCTIONAL AGRICULTURE

Z. BEDŐ

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The concept of the sustainable development of agricultural production marked the beginning of a new era in agriculture worldwide. The term sustainability was first interpreted primarily as the sustainability of the environment, due to the ever more serious problems experienced in this connection on a global scale. In searching for a solution, however, focus shifted to a complex approach to sustainable development. It became clear that in addition to the sustainability of the environment, a long-term solution could only be achieved if economic and social sustainability was also ensured. This is particularly true of agriculture, since the existing problems cannot be solved purely on the basis of environmental considerations. Only the comprehensive handling of ecological, economic and social challenges can produce a satisfactory answer to the questions involved in sustainable development. This will necessarily mean a change in the tasks facing agriculture, which will be responsible for more numerous, more varied functions than previously. If these new tasks are to be successfully performed, new technological systems will need to be elaborated, requiring an acceleration of research and development throughout the world.

Key words: crop production, multifunctional agriculture, sustainable development

Introduction

If multifunctional agriculture becomes a reality, it will influence the development of all sectors of agriculture. Crop production will be affected perhaps to the greatest extent, since it is in direct contact with the natural environment and its functions and technological systems have a considerable influence on sustainable development.

In recent years the intensive development of crop production, a process which became known as the green revolution, was witnessed throughout the world. Intensive crop production evolved, involving an increase in plant productivity and the widespread use of chemicals, machinery and irrigation. The breeding of up-to-date plant varieties and the spread of industrial technologies helped to solve a problem which has existed throughout mankind's history: famines were largely overcome and food shortages were eliminated on a global scale.

Since the last decade of the twentieth century it can be justly said that the malnutrition of the population in certain parts of the world is due chiefly to the continued existence of bad social or economic distribution systems and to the underdeveloped infrastructure, and only to a small extent to deficiencies in food

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production. This is particularly true of Europe. On a global scale the world's agriculture is capable of producing sufficient food for mankind, despite the fact that the world population has exhibited an unprecedented increase over the last half century.

Food production, however, is unevenly distributed over the countries of the world. This means that food shortages and overproduction are present at the same time in different regions of the world, so there are simultaneous calls for an increase and a decrease in the sowing area, and for the further development or reduction of agriculture. This wide spectrum of differing concepts is the result of the existing differences in the state of development and in the natural potential, which are in most cases also to be found within each individual country.

Crop production in multifunctional agriculture

Nowadays more and more attention should be paid to agricultural problems involved in rural development, to promoting the healthy nutrition of the population and to developing a multifunctional approach to agriculture, capable of maintaining the equilibrium of the natural environment. All three fields will play an important role in the quality of human life in the society of the future. It follows from this that the social importance of multifunctional agriculture in the future will depend not so much on its share of the gross national product, as on its role in determining the everyday living standards of the population. In this new situation it will be more important for crop production to produce raw materials of a quality capable of preserving or improving the health of the consumer, at the same time providing sustainable development for the rural population and contributing to the preservation of the traditions and special features of the different regions, while not endangering the ecological balance of nature.

Several different concepts on the future prospects of crop production have evolved, each with its own technological system. These can be grouped fundamentally in the intensive and/or high input category or in the extensive and/or low input category, but within this general grouping there are numerous variants and subgroups, aimed at adapting to regional conditions. Despite the pronounced differences in approach, each concept attempts to elaborate a technological system which will satisfy food production expectations and thus gain the approbation of the general public. The preconditions for this are:

- the use of technologies which protect the environment;
- the sustainability of production with the given technology;
- the production of nutritious food free of substances endangering human health.

When examining the sustainability of crop production it is of fundamental importance to ensure that the technological system introduced in any part of the country should, whether intensive or extensive, suit the agroecological needs of the region in question. Any type of technology may be successful and may contribute to sustainability if

• the production technology can be integrated into the multifunctional crop production system and is suited to the agroecological potential of the growing region, and

• the consumers are prepared to accept and purchase the products thus produced.

If both of these conditions are fulfilled it is an indication that the system is satisfactory from the ecological, economic and social points of view and could thus be a factor in long-term sustainable development.

Characteristic features of Hungarian crop production in multifunctional agriculture

The large proportion of the land area taken up by crop production in Hungary is unusual on an international scale. This large territorial ratio, which has not changed to any great extent since the change of regime, gives added justification for the development of a multifunctional crop production strategy, in which regional differences are taken into account. Serious problems could be expected within a short time if a uniform technological system were implemented throughout the country without a knowledge of local agroecological conditions.

It is well worth giving some thought to the technological concepts elaborated in Denmark, where the crop production situation is similar in many respects. In Denmark crop production is carried out on some two-thirds of the country's territory, and thus has a decisive effect on the ecological balance in the whole country. As so much is at stake, some of the firmest proponents of multifunctional agriculture are to be found in Denmark. The term multifunctional agriculture, however, covers everything from organic farming free of all chemicals to low input technologies and precision agriculture, depending on what production system provides the best guarantee of sustainability in the given region.

Table 1

Land occupied by various branches of agriculture in Hungary, in thousand hectares (data from the Central Office of Statistics)

Branch of agriculture	1980	%	1990	%	1999	%
Arable	4734.7	50.9	4712.8	50.6	4708.0	50.6
Horticulture	291.4*	3.1	341.1*	3.7	107.7	1.1
Orchards	138.4	1.5	95.1	1.0	96.4	1.2
Vineyards	167.8	1.8	138.5	1.5	127.1	1.3
Grassland	1294.2	13.9	1185.6	12.7	1147.1	12.3
Total agricultural area	6626.5	71.2	6473.1	69.5	6186.3	66.5
Forests	1610.3	17.3	1695.4	18.2	1774.9	19.0
Reed-beds	37.7	0.4	40.3	0.4	41.1	0.4
Fish farms	25.3	0.3	26.9	0.3	32.8	0.5
Total production area	8299.8	89.2	8235.7	88.5	8035.1	86.4
Non-cultivated area	1003.8⁺	10.8	1067.5⁺	11.5	1267.9	13.6
Total land area	*9303.6*	*100.0*	*9303.2*	*100.0*	*9303.0*	*100.0*

*Including private gardens ⁺Excluding private gardens

Field crop production data for Hungary over the last three decades indicate that the arable area has remained relatively constant at around 4.6–4.8 million hectares (Table 1). The change in structure has thus not involved a reduction in the crop production area. On the other hand, there has been a clearly perceptible drop in the territorial proportion of orchards and vineyards. Notwithstanding, some two-thirds of the country's land area was used for agricultural production at the turn of the century.

The changes in the sowing areas of major crops reflect the structural changes which have taken place in arable crop production. This process was not uniform over the last decade; large fluctuations were witnessed and in some years substantial areas were not cultivated. The situation for the main groups of crops can be summarised as follows (Table 2):

- although there were considerable fluctuations, the sowing area of cereals only declined to a slight extent, if at all;
- the area sown to oil crops increased to the greatest extent;
- the sowing area of fodder crops decreased due to the reduction in livestock farming;
- the area sown to protein crops continued to stagnate at a low level or to decline.

The total area sown to cereals has not changed substantially, but the spread of triticale is a good example of the fact that farmers were happy to grow this new species, mainly due to its low pesticide and fertiliser requirements. In spite of the fact that the production of fodder crops declined, the sowing area of triticale rose from zero to a hundred thousand hectares.

Table 2

Mean annual sowing area of major field crops in Hungary, in thousand hectares (data from the Central Office of Statistics)

Crop species	1979–81	1989–91	1998–2000
Winter wheat	1187	1205	989
Winter barley	113	191	151
Spring barley	151	121	196
Rye	72	94	49
Oats	45	48	61
Triticale	–	–	102
Maize	1248	1091	1213
Sunflower	268	364	416
Rape	47	59	116
Alfalfa	370	301	197
Red clover	53	20	9
Peas	91	136	43
Soya beans	25	40	26
Sugar beet	113	137	68
Potatoes	67	45	52
Vegetables	118	111	103

As far as the future is concerned, the development of multifunctional crop production may lead to considerable structural changes as regards both the various branches of agriculture and within the field crop production sector. It would certainly be an advantage to increase the number of crops grown, both in order to improve biological and production diversity, and to ensure safer marketing openings.

Plant varieties for multifunctional crop production

The introduction of new plant varieties into cultivation is an integral part of the technological system of crop production. If they are to satisfy the requirements of multifunctional agriculture, plant breeders will have to face much more complex challenges than previously. Far more attention will have to be paid to

- the relevant crop production technology,
- the demands of the processing industry,
- consumer reactions to the plant species or variety,
- social and cultural habits and traditions in the field of nutrition.

The extent to which various plant species will spread in the future will depend not only on the climatic conditions, but also on ecological, economic and social criteria. This means that the quality of the interaction with the agroecological environment will become an important precondition for production. Among other things this will involve a consideration of how various crops influence the quality of the natural environment or the soil in the neighbourhood in which they are grown. Society will force farmers to rethink the production conditions of crops having a negative influence on the natural environment and, if necessary, to remove them from cultivation.

The multifunctional character will become an important criterion not only for production technologies, but also for plant species. The more processing purposes a plant species can be used for, the larger area it can be economically grown on. The classical requirement if a species was to be grown on a wide scale was for it to "stand on three legs", being suitable for

- human consumption,
- animal feeding, and
- industrial processing.

Thanks principally to molecular breeding and biochemical research, the possibilities for utilisation have expanded and in addition to traditional processing quality scientists are now developing new quality types in many plant species. This means that the purposes for which different varieties of a plant species are cultivated may be widely different, requiring the elaboration of quite different production technologies.

In earlier years the primary goal of plant breeders was to improve yield potential and to develop varieties that satisfied the requirements of growers. This is no longer the case. The criteria to be met in breeding new varieties have now become more complex. One example of this is the complexity which characterises the acceptance of new plant varieties. Today the choice of variety depends not only on the yield potential, but also on other characters and conditions. Importance is now attached to

- the opinion of the processing industry,
- the willingness of retailing chains to market the product and, even more importantly,
- public opinion, or the trust of the consumer.

Nowadays the consumer has the right of veto in all questions, having a decisive influence on the opinions expressed by traders and processors. If the consumer, for instance, refuses to buy foodstuffs made using genetically modified plants, the consequences of this decision will affect the grower, so this is of great importance in the choice of variety and technology. Until it proves possible to convince consumers of the advantages of transgenic plant varieties, it will be extremely difficult to extend the area sown to genetically modified plants in Europe. The herbicide tolerance of a transgenic plant may be an enormous advantage from the production point of view, but if the consumer is indifferent to this or draws negative conclusions from this fact, the transgenic plant will not be cultivated.

If transgenic plants are to gain ground in Europe it will be necessary to satisfy consumer expectations and win the confidence of the consumers. Once molecular breeding has overcome this obstacle, the seed industry will be able to market the seed of genetically modified plant varieties all over the continent and these varieties will then also be commercially grown in Hungary. The use of molecular methods in genetics and breeding will make a great contribution to the development of multifunctional agriculture, despite the fact that transgenic varieties will most probably be used first in precision or intensive crop production technologies.

Over a five-year period from the mid-nineties the area sown to genetically modified plants rose to around fifty million hectares worldwide. Approximately half this area is sown to soya beans, while transgenic maize is grown on over 10 million hectares, cotton on over 5 million hectares and rape on about 3 million hectares. The vast majority of the transgenic plant varieties presently cultivated contain genes responsible for herbicide tolerance or Bt insect resistance.

Technology systems in multifunctional crop production

The subsidy system of the EU attaches fundamental importance to environmental protection criteria and to the maintenance of the ecological equilibrium in the natural environment. Multifunctional agriculture will thus make itself felt in the day to day life of the farmers in the form of the subsidy system. The regional distribution of areas suitable for agriculture into three groups and the stipulation of what technologies can be applied on them also provides a basis for multifunctional agriculture. These groups are as follows:

- areas suitable for agricultural production;
- ecologically sensitive areas where environmental protection criteria take precedence over agricultural production;
- areas removed from agricultural production.

It has been estimated that some 60–70% of the agricultural area in Hungary can be put in the first group, with some 20% in the second and 10% in the third.

On areas belonging to the first group the development of crop production technologies suitable for competitive agriculture holds out the best prospects. Farmers in these regions will grow crops chiefly using intensive technologies. In addition to the present technologies, precision crop production technologies have been introduced widely in developed countries, the advantages of which can best be exploited on large farms. The acceleration of land and farm concentration in Western European countries can partly be attributed to the change in technology.

The use of transgenic plants could be of great significance in precision crop production technologies. Due to the first results achieved with gene technology the sowing area of transgenic plant varieties has increased in North America, China, Argentina and in more and more developed and developing countries. This, however, is not true of Europe, even though the cultivation of genetically modified plants bears enormous potential for scientifically-based agricultural production. They could be applied extremely profitably both in precision farming and in low input technologies, where they would allow the use of chemicals, such as pesticides, to be reduced and the stability of production to be enhanced. All these potential advantages could be efficiently exploited in the future even in less intensive technologies. However, this will require a considerable change of attitude, which would be greatly promoted if environmentalists gave up their negative opinions.

The various types of low input technologies, organic farming and biofarming which have evolved in recent years represent a different approach to sustainable development, the importance of which is increasing at present, especially in Western Europe. The increasing consumer demand for bioproducts should not be ignored, as this has had a positive effect on prices. There is a definite need for a larger proportion of organic farming in the competitive sector too. A distinction must be made between organic farmers working in a competitive environment and satisfying the requirements of an ever widening group of consumers and those who carry out biofarming on a social basis or as a hobby. The climate of Hungary, which is prone to drought, is excellent for ecofarming in the competitive sector, since good quality bioproducts can be grown with fewer chemicals than in Western Europe.

The ecologically sensitive areas include poorly fertile territories which are less suitable for crop production. As agriculture is less profitable, farmers need subsidies, which should be part of an up-to-date rural development system. In the majority of cases the main aim is not to make farming profitable, but to retain the population and to provide jobs, at the same time preserving the special features of the landscape and the ecological equilibrium. Environment protection considerations have priority when choosing the crop production technologies, so these areas are less suitable for competitive crop production. Instead the emphasis should be on production for local consumption, village tourism, etc.

It is quite clear that within agriculture as a whole, crop production has developed enormously over the last half-century. However, a new era is again beginning, and real changes will come rapidly when Hungary joins the European Union. Developments in crop production will depend on how successfully it becomes integrated in multifunctional agriculture. Efficient farming will only be possible if the organisational structure and production technologies are adapted to suit consumer demands in a situation far more complicated than in earlier years. It is quite certain that the large production volumes achieved in earlier decades will not be seen again. Instead of quantitative indexes, quality and efficiency parameters will determine whether the technological systems applied in Hungarian crop production can be regarded as sufficiently up-to-date.

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Review

CROP PRODUCTION RESEARCH IN MULTIFUNCTIONAL AGRICULTURE

Z. BERZSENYI

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The research agenda for crop science in the 21st century will depend largely on whether the present conditions regarding the global food surplus continue, or whether a food scarcity recurs. Crop production research is based chiefly on small-plot field experiments, the majority of which are either long-term experiments or experiments set up to investigate the specific agronomic responses of Martonvásár maize hybrids and wheat varieties. The sustainability of crop production is examined in long-term experiments. The agronomic responses of maize hybrids and wheat varieties are studied at various levels of biological organisation. Growth analysis facilitates the exact characterisation of agronomic responses and the grouping of response effects and types using multivariable methods. Continued experimentation coupled with crop simulation models and decision support systems are an ever more useful framework for analysing the complexity of agricultural systems.

Key words: maize, wheat, long-term experiments, agronomic responses, plant growth analysis

Introduction

Science, technology, environmental changes and the world population are the most important human and environmental factors which will cause changes in agricultural systems over the next few decades. These changes are expected to be far-reaching for a number of reasons. In the first place mention should be made of the continuous, intensive use of science and technology, especially biotechnology and information technology. Secondly, there has been a gradual recognition of the fact that industrial-type crop production systems may cause considerable damage to the environment and that only the development of sustainable agricultural systems will bring a solution. Finally, the least predictable of these factors is the effect of the climatic changes which are likely to occur due to global warming.

Research priorities in crop production

Crop production research in the 21st century will depend to a great extent on whether the present global overproduction of foodstuffs continues or whether food shortages are again experienced. The accurate prediction of food supplies and demands is thus a key question in the outlining of research priorities

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(Cassman, 2001). If there continue to be food surpluses research priorities will include the reduction of production costs, an increase in the end-use value of plant products and the diversification of production systems, in order to minimize over-production. The aim of strategic research on the improvement of production efficiency is to determine not so much the marginal returns on various resources, but the minimum level of each production resource which will allow the maximum utilisation of all the other resources.

In order to achieve site-specific cultivation technologies, accurate data will be required on spatial and temporal changes in soil properties, in the physiological status of the plant and in pests and pathogens, and on how the plants respond to these changes. Our present understanding of the ecophysiological processes regulating plant responses is not sufficient, however, for a precise prediction of site-specific input requirements or of the result of their application. This lack of knowledge is the most important factor limiting the large-scale adaptation of site-specific cultivation techniques in agriculture.

In the case of a global food shortage, greater priority will be given to increasing the yield potential of cereals and the average farm yields in major cereal-producing systems. The dual aim of maintaining yields at a level approximating to the maximum attainable yield and of reducing negative environmental effects will become critical if there are food deficiencies. If yield increases are to be achieved in less favourable environments, research must be focussed on yield optimisation, yield stability, input utilisation efficiency and profitability under extremely changeable climatic conditions.

Aims of crop production research and types of experiments

Crop production research in Martonvásár is based largely on small-plot field experiments. The backbone of the research consists of long-term basic experiments and of tests on the specific agronomic responses of the maize hybrids and wheat varieties bred in Martonvásár. The agroecological approach is emphasised in this research, which thus concentrates not just on yields but also on the ecological sustainability of the production system. Three types of small-plot field experiments are used in crop production research: (a) long-term experiments, (b) response-predicting experiments and (c) experiments on the adaptation of new technologies.

In the course of crop production research the agronomic responses of maize hybrids and wheat varieties are investigated at various levels of plant organisation (plant stand, individual plant and plant organ). Biotic and abiotic factors are measured at the agronomic level in terms of grain yield, yield components, total biomass and biomass allocation at physiological maturity. At the plant stand and individual plant level the responses of maize hybrids and wheat varieties are measured over periods of days or weeks throughout the life cycle and the genotype × developmental phase interaction is examined. In addition to quantifying differences in yield between the genotypes, this research is also aimed at determining the factors responsible for these differences. As well as measuring the direct effect of various factors, ever greater importance is attached to analysing the interactions between two or more factors in various types of multifactorial experiments.

Sustainability of crop production

The three most important indicators of the quality of an agroecosystem are productivity, stability and sustainability. One of the major challenges in the 21st century is to achieve sustainable ecosystems. Sustainability can only be measured over a long period of time, so 10–20 years are usually required before trends in the sustainability of a system become obvious. Sustainable production is characterised by: (1) a non-declining trend in yields and in the productivity of all production factors, (2) satisfactory yield stability and (3) an adequate level of all factors indicating the quality of the ecosystem. The measurement over time of yield stabilty, which is an important indicator of sustainability, involves at least three components: (a) the dependence of the yield on the local environment, (b) the mean yield level and (c) the variability in yield. A stable system can be defined as one which hardly changes as the result of environmental effects.

In Martonvásár the sustainability of crop production technologies is examined in long-term experiments. It is only from such experiments that satisfactory indicators (yield trends, parameters characteristic of the quality of the ecosystem) can be obtained on the sustainability of production, thus enabling them to serve as an early warning system. The long-term crop production experiments set up over 40 years ago by Dr Béla Győrffy, which were the first in the country, are living laboratories of enormous value for research, education and the extension service. Numerous papers have already been published on the results of the long-term experiments (e.g. Berzsenyi and Győrffy, 1996; Árendás et al., 1998; Berzsenyi et al., 2000). The use of discriminant analysis to discriminate between wheat crop rotations in long-term experiments is illustrated in Figure 1 (Berzsenyi and Lap, 2002).

Yield potential, yield stability and stress tolerance

The main factor determining the potential yield is the climate (temperature, solar radiation), while the attainable yield also depends on the availability of water and nutrients. The actual yield, at field level, is further influenced by many biotic and abiotic effects and by economic, environmental and social factors. While the temperature and solar radiation represent the final limits of the potential yield, the achievement of this potential also requires a high input of resources and the efficient utilisation of these resources. A knowledge of the yield level for a given variety, location and year makes it possible to examine the extent to which the actual yield falls short of the potential yield and how the yield could be further increased.

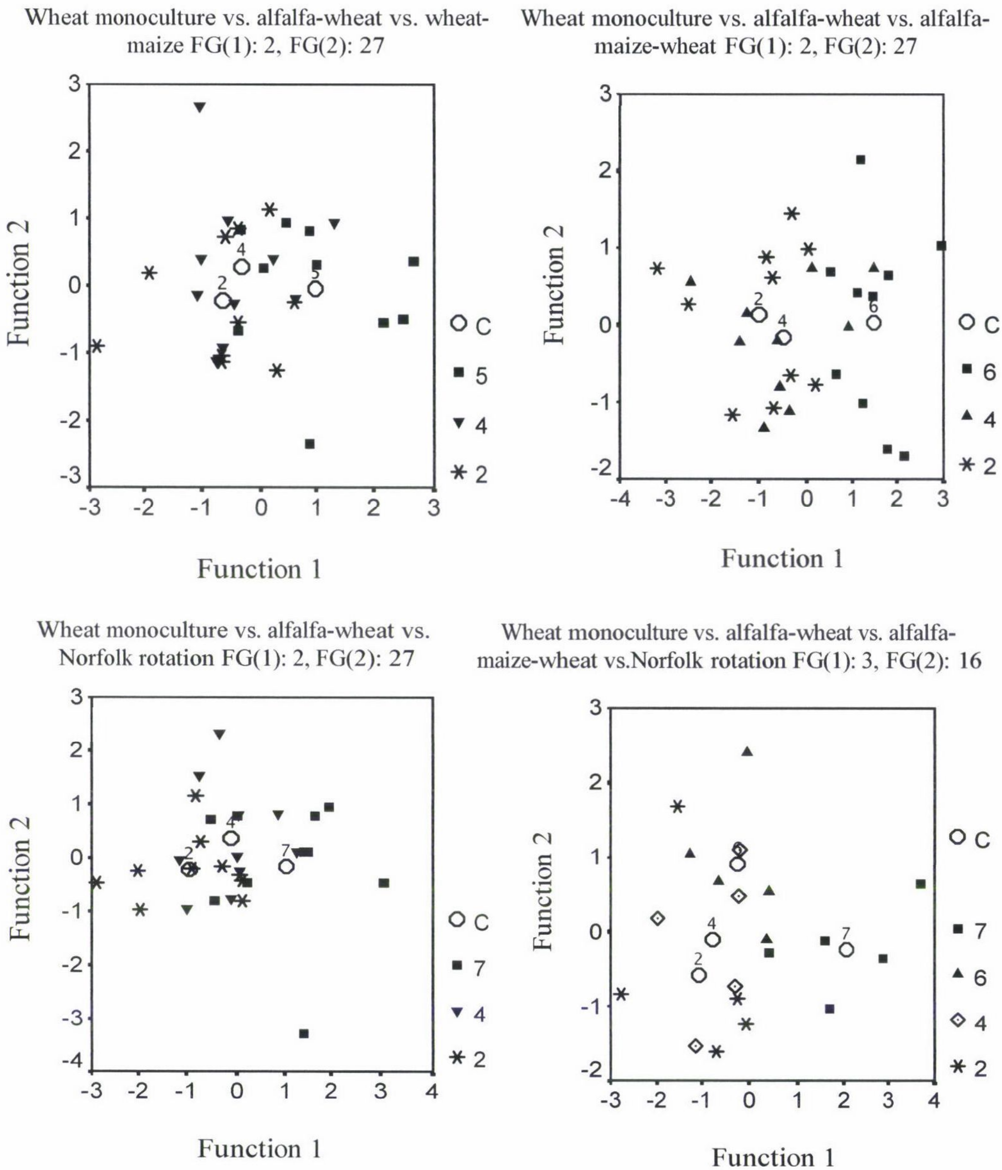

Fig. 1. Multivariate separation of wheat crop rotations vs. monoculture using discriminant analysis. Legend: C: Group centroids, 2: Wheat monoculture, 4: Alfalfa-wheat, 5: Wheat-maize, 6: Alfalfa-maize-wheat, 7: Norfolk rotation

Increases in maize and wheat yields are the result of genetic, agrotechnical or ecophysiological changes which are affected by short- and long-term climatological changes. Yield increases are traditionally attributed to genetic gain and agrotechnical development. Recent research has demonstrated that a considerable proportion of the yield increase is a consequence of the genetic × agrotechnical interaction. Based on 35 years of data from the Martonvásár long-term experiments, crop production factors have made the following contributions to the yield increase of maize (%): fertilisation: 30.7, variety: 30.0, plant density: 20.3, weed control: 16.3, deep tillage: 2.7. These figures also include the interactions (Berzsenyi and Győrffy, 1995).

Maize and wheat yield increases are related to an improvement in stress tolerance, which is partly the result of breeding for greater yield stability. Yield stability is the ability of the genotype to maintain the same relative yield under widely different environmental conditions. Crop production research is in progress to determine the effect of various agronomic (crop rotation, fertilisation, sowing date, plant density) and ecological (e.g. year effect) factors on maize and wheat yields and on yield stability. Figure 2 illustrates the effect of minimum and optimum levels of crop production factors on the yield stability of maize in long-term experiments on the basis of the Finlay-Wilkinson (1963) model (Berzsenyi and Győrffy, 1995; Berzsenyi and Lap, 2001).

Environmental and agronomic responses of crops

The environmental responses of the crop determine its adaptation and influence the improvement of crop production systems through agronomic development and breeding. This aspect is important because the efficiency of crop production will have to be increased substantially in the 21st century. An understanding of the environmental responses of plants is fundamental for the elaboration of the methods required to increase efficiency.

If plant responses are to be understood, information is required from various levels of biological organisation. Research is necessary at higher levels of organisation if we are to understand the effect of changes in the variety or agrotechnical measures on plant productivity or on other responses of the plant stand. This is due to the fact that the various levels are hierarchical and the higher levels of organisation also have uniquely important facets. Functions at the plant stand level determine whether the productivity of the crop will increase as the result of changes in the variety or the cultivation techniques.

Ever greater efforts are being made in crop production research not only to measure the effect of experimental treatments in terms of the end-product (grain yield, biomass), but also to trace changes in the dynamics of photosynthetic production throughout the growth period of the plants. Growth analysis, which is fundamentally an ecological method, is especially suitable for the comparative analysis of the growth of crops and of the ecological and

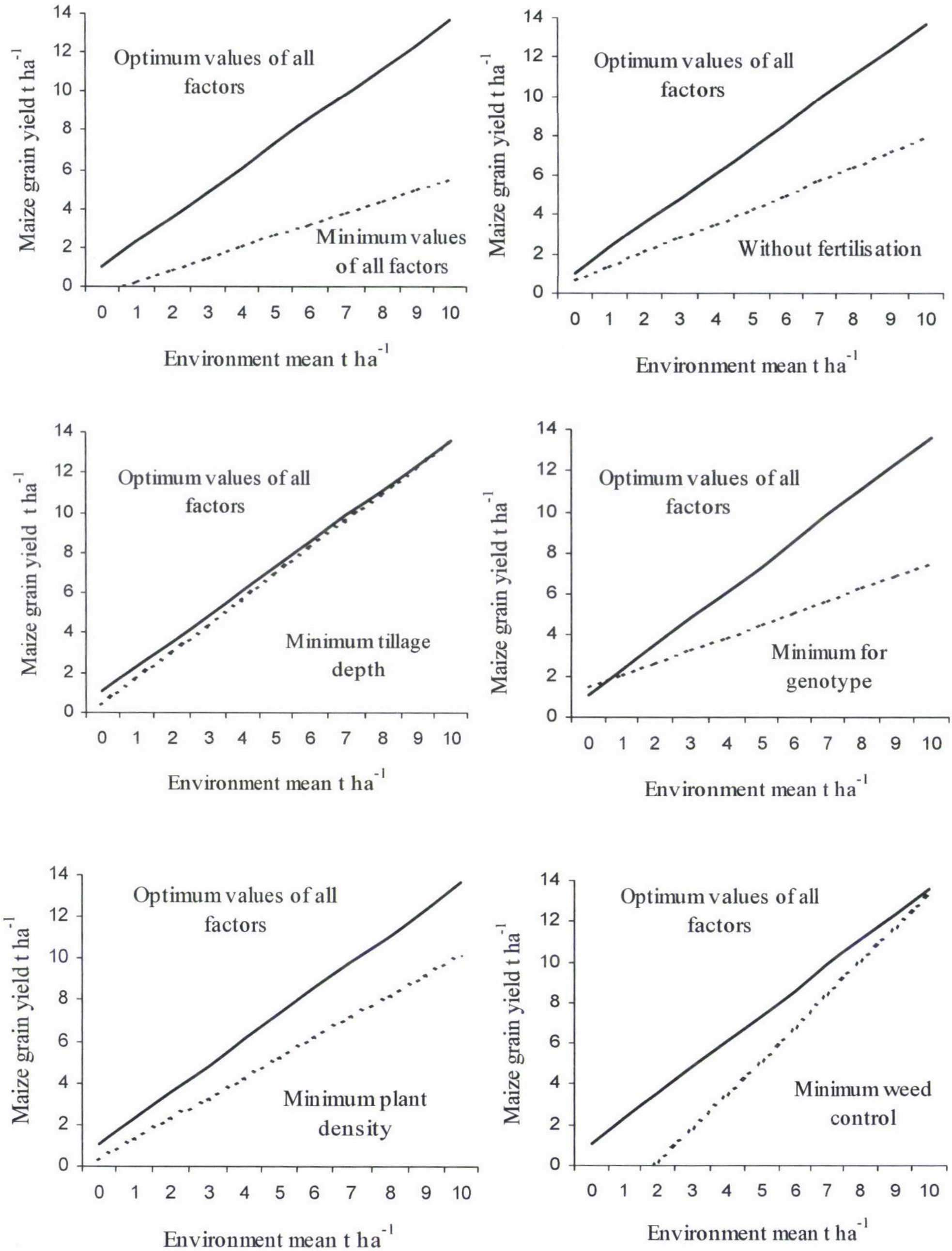

Fig. 2. Effect of minimum and optimum levels of crop production factors on the yield stability of maize in long-term experiments (1961–2000)

agronomic factors influencing this growth. The aim of the research is to understand the biological background of yield formation at the plant stand, individual plant and plant organ levels. In this way growth analysis leads to the accurate characterisation of agronomic responses and the multivariable discrimination of the magnitude and type of response. The fact that the results promote a better understanding of physiological and ecological processes, thus contributing to efforts to increase yields, has given this research added momentum. The results achieved in growth analysis research aimed at giving a clearer picture of the agronomic responses of maize hybrids and wheat varieties have been published in a number of papers (e.g. Berzsenyi and Lap, 2000). The effect of genotype, sowing date, plant density and N fertilisation on the absolute growth rate (AGR) of maize dry matter production is illustrated in Figure 3. The relevant research has consistently proved a close correlation between AGR and grain yield.

Crop simulation models and decision support systems

The advantages of integrating crop simulation models into research programmes can be summarised as follows (Matthews et al., 2002): (1) the pinpointing of gaps in present knowledge, (2) the generation and testing of hypotheses to promote improvements in experimental design, (3) the determination of the factors with the greatest influence on the system (sensitivity studies), (4) the forging of links between various research disciplines, (5) the promotion of cooperation between scientists and growers in order to solve common problems.

The models are of especial value in synthesising various interpretations of experimental results and in integrating reductionist research approaches. If the efficiency of research is to be improved, the modelling process must become an integral part of research activities. Experimentation and model development must advance hand in hand, utilising new knowledge to perfect or improve the models and applying the models to identify gaps in our present knowledge and to delineate research priorities. Together, continuous experimentation, monitoring and simulation models represent an efficient approach to an understanding of the interactions between the plant, the soil, the weather and crop production factors, thus promoting the sustainability of production systems.

In addition to using the models in research, numerous attempts have been made in recent years to use crop models to assist farmers in making decisions, leading to the setting up of decision support systems (DSS). The aim of the IBSNAT project (Tsuji et al., 1998) is to make the knowledge accumulated in the course of research and technology development available to users, thus serving the triple purpose of research: the understanding, prediction and regulation of the processes and mechanisms involved in agroecosystems. Modelling is likely to become an increasingly important method in research on the sustainability of production and on environmental problems for the simple reason that there is often no other approach capable of quantifying these complex processes.

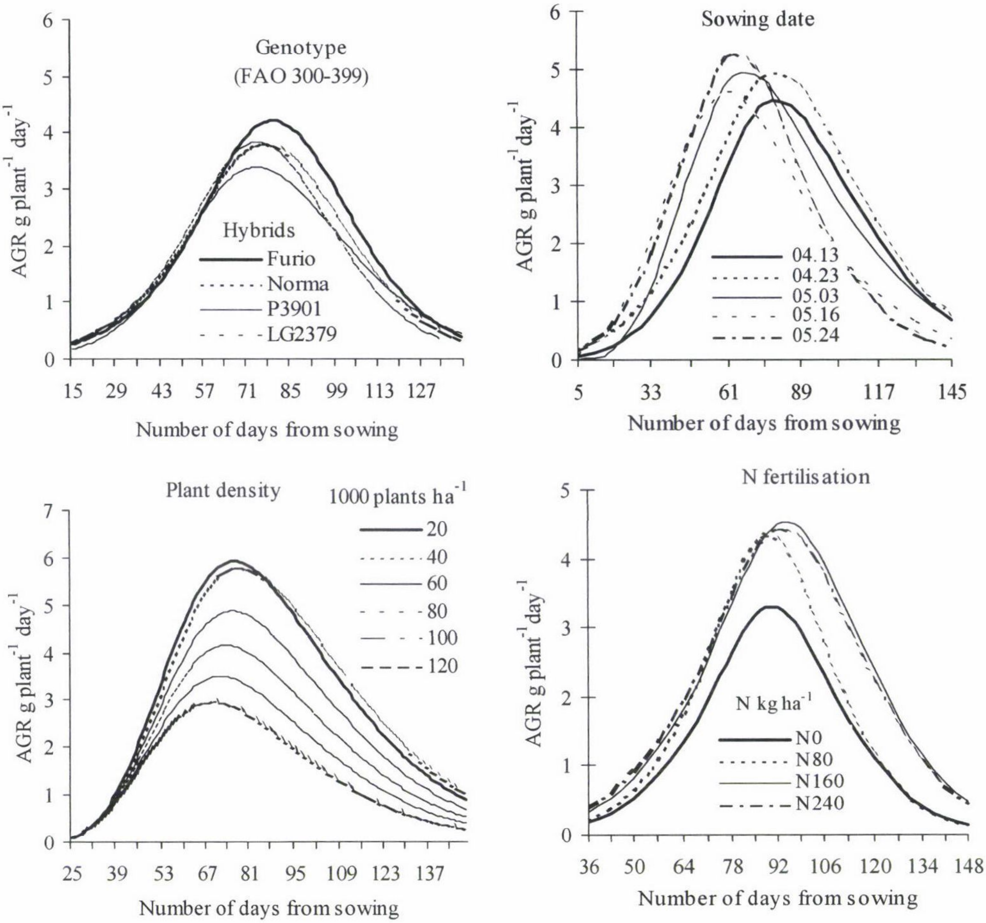

Fig. 3. Effect of genotype, sowing date, plant density and N fertilisation on the absolute growth rate (AGR) of maize dry matter production

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Review

IMPORTANCE OF AGRICULTURAL CHEMISTRY IN MULTIFUNCTIONAL CROP PRODUCTION

T. NÉMETH

RESEARCH INSTITUTE OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY

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Introduction

Agricultural production is a basic, traditional constituent of the Hungarian economy. An importance question nowadays is how the land can be cultivated and agricultural goods produced under the conditions of sustainable development.

From the plant nutrition point of view the establishment of an environment-friendly fertilizer recommendation system is essential if sustainable development is to be achieved. Most experts agree that this type of fertilizer recommendation system is able to fulfil the growing demands of a growing population, while keeping the environment in good condition for the next generations. The experts also agree that fertilizer application could not be replaced widely with organic farming alone.

An environmentally friendly fertilizer recommendation system has to be sensitive enough to respond to the effects of different conditions, e.g. great spatial variability of soil characteristics, mosaic-like soil cover, climate, crop rotation practices, soil nutrient supply, etc.

There was a dramatic change in Hungarian agriculture at the beginning of the 90s, as the result of which the use of fertilizers decreased sharply for several reasons, e.g. privatization, changes in ownership, withdrawal of state subsidies for mineral fertilizers, drought, etc.

Both Hungarian agriculture and the country as a whole is now facing two challenges, i.e. to overcome the economic difficulties and to complete the final phase of preparations to join the EU. Land use change scenarios have proved that the natural endowments of Hungary are suitable for integrating agricultural production with environmental and landscape protection and nature conservation.

Fertilizer consumption

Under the terms of sustainable agricultural development it is not enough for the fertilizer application to result in yield increases and profit. From the crop production point of view, in order to achieve a reliably high yield with good

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quality, special attention should be paid to satisfying the nutrient demands of the crops. To this end organic or mineral fertilizers should be applied over a certain yield limitation. Whether the fertilizers are needed to keep (or in some cases increase) the yield, or to maintain (or increase) the fertility level of the soil, the farmers must apply a certain amount of the right kind of fertilizer (Cooke, 1992).

From the early 60s till the late 80s in many countries, including Hungary, agricultural production increased tremendously. In Hungary the yield of cultivated crops doubled or even tripled because of the introduction of intensive agricultural practices (IAP). Several factors contributed to this impressive yield increase, such as

- the introduction of new high-yielding varieties of crops,
- the improvement (or maintenance) of the nutrient supply (fertility) of the soils by applying fertilizers,
- plant protection,
- mechanization.

During this period the use of mineral fertilizers multiplied from an annual 168,000 tons (1960) up to 1,586,000 tons (1983). The change in fertilizer application can be seen in Table 1. The intensive application of fertilizers improved the fertility of the cultivated soils, so that nutrient balances became positive. The retention of these positive nutrient balances for over 20 years resulted in the NPK enrichment of the soils, as proven by several national soil test series. This is why in the late 80s the aim was simply to keep and maintain these fertility levels (Németh, 1993; Sarkadi and Várallyay, 1989; Várallyay et al., 1992). The fertilizer application data show a dramatic change from the early 90s. For several reasons, e.g. privatization, changes in ownership, withdrawal of state subsidies for mineral fertilizers, drought, etc., the use of fertilizers decreased sharply (Csathó and Németh, 1996). There is no doubt that during the intensive period of Hungarian agricultural development some cultivated soils were overfertilized, but the decrease was out of all proportion to the former overdosage. The previous oversupply and the dramatic change, which was not brought about by developments in agriculture, aroused interest in elaborating an environment-friendly fertilizer recommendation system. Such a system should be sensitive enough to respond to the effects of different conditions, e.g. great spatial variability of soil characteristics, mosaic-like soil cover, climate, crop rotation practices, soil nutrient supplies, etc. Taking these conditions into consideration a computerized fertilizer recommendation system was developed in the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences (RISSAC) to suit the soil fertility levels in different regions of Hungary under diverse agricultural practices and environmental conditions (Németh, 1996b; Várallyay et al., 1992).

Table 1

Farmyard manure (million t $year^{-1}$) and fertilizer use in Hungary, 1931–2000 (Statistical Yearbooks for Agriculture, Central Bureau of Statistics)

Year	Farmyard manure	Fertilizer active ingredients, 1000 t $year^{-1}$				For arable lands, kg ha^{-1} $year^{-1}$
		N	P_2O_5	K_2O	Total	
1931–40	22.4	1	7	1	9	2
1951–60	21.2	33	33	17	83	15
1961–65	20.6	143	100	56	299	57
1966–70	22.2	293	170	150	613	109
1971–75	14.8	479	326	400	1,205	218
1976–80	14.3	556	401	511	1,468	250
1981–85	15.4	604	394	495	1,493	282
1986–90	13.2	559	280	374	1,213	230
1991–95	6.0	172	25	26	223	44
1996–2000	4.8	235	40	42	317	63

Environment -friendly fertilizer recommendation system

During the elaboration process the soil of all the agricultural land was classified using a 4-digit code system according to its characteristics (Németh, 1996b; Sarkadi and Várallyay, 1989; Várallyay et al., 1992). The first digit of this new land-site category system reflected the soil water regime and climate for the soils without any fertility limitation processes:

1 – chernozem soils (migration type),
2 – brown forest soils (leaching type),
3 – meadow soils (hydromorphic type).

(There are 5 other land-site categories each reflecting one of the fertility limitation characteristics, like salinization, skeletal soils, etc.)

The second digit for land-site groups 1–3 reflects the soil texture, the third the soil reaction (pH) and $CaCO_3$ content, and the fourth the organic matter status.

Altogether 280 existing soil mosaics could be distinguished in Hungary on the basis of this classification system. In the next step the existing soil mosaics were classified into aggregated land-site groups, using a matrix table compiled by specialists taking into consideration the major plant nutrients. Six aggregated groups were elaborated for N, P, K and Mg, and three for Ca. Within these aggregated land-site groups, the nutrient status of the soil was characterized by a "nutrient supply" category. For nitrogen this was based on the organic matter content of the soil, while for P and K it was based on the AL-soluble P_2O_5 and K_2O contents, respectively. The system uses five nutrient supply categories for each of the elements (NPK), from 1 (low) to 5 (high); thus 30 (6×5) units exist for each of the macronutrients (Table 2).

Table 2
Nutrient supply categories for aggregated land-site groups

Aggregated land-site groups	Supply categories				
	very poor	poor	medium	good	very good
For N (based on OM %)					
I	< 1.0	1.0–1.7	1.8–2.4	2.5–3.0	> 3.0
II	< 1.5	1.5–2.0	2.1–3.0	3.1–3.5	> 3.5
II	< 2.0	2.0–2.5	2.6–3.5	3.6–4.0	> 4.0
IV	< 2.5	2.5–3.0	3.1–4.0	4.1–5.0	> 5.0
V	–	–	–	5.0–15.0	–
VI	–	–	–	> 15.0	–
For P (based on AL-P_2O_5 mg/kg)					
I	0–30	31– 60	61–100	101–180	181<
II	0–40	41– 80	81–120	121–200	201<
II	0–50	51–100	101–150	151–240	241<
IV	0–60	61–120	121–170	171–270	271<
V	0–70	71–150	151–200	201–300	301<
VI	0–80	81–180	181–250	251–400	401<
For K (based on AL-K_2O mg/kg)					
I	0–50	51– 80	81–120	121–160	160<
II	0–60	61–100	101–150	151–200	200<
II	0–80	81–120	121–180	181–250	250<
IV	0–100	101–150	151–210	211–280	280<
V	0–120	121–180	181–250	251–320	320<
VI	0–150	151–210	211–280	281–350	350<

The fertilizer requirements of the crops, on the basis of specific nutrient contents [N, P_2O_5 and K_2O (kg/ha) needed for the production of 1 t yield] are also taken into account for each of the 5 soil nutrient supply categories set up for N, P and K. The system was elaborated in such a way that the specific nutrient contents, modified according to the nutrient supply category, must be multiplied by the expected yield (i.e. for 5.5 t/ha winter wheat this amount has to be multiplied by 5.5 for each of N, P and K).

Nutrient balances in Hungarian soils

In the period preceding World War II the nutrient status of Hungarian soils was poor due to the negative nutrient balance resulting from yield exports with minimal recycling of plant residues, organic or green manures, and mineral fertilizers. From the turn of the century till the late 50s, nutrient balances in Hungary were strongly negative: 20–30 kg/ha/year less N and K_2O and 10 kg/ha/year less P_2O_5 was given to the fields in different forms than was removed with the harvested yields (Table 3). The nutrient balance for phosphorus became positive in the early 60s, and the balances of nitrogen and potassium in the 70s. For the next 20 years, the N balances were positive by 10–20 kg/ha a year and both the P_2O_5 and K_2O balances by 30–50 kg/ha. From the early 90s, however, the dramatic decrease in fertilizer use resulted in changes in the nutrient balances as well: in 1991 the N balance was –60, the P_2O_5 balance –30 and K_2O balance –40 kg/ha for the whole of the arable land. From this year onwards the balances remained negative.

Table 3
Average nutrient balances in Hungarian soils, 1932–1991 (kg/ha agricultural lands)
(Kádár, 1979, 1987, Csathó, 1994)

Items of balance	1932–36	1960–64	1971	1975	1985	1990	1991
				Nitrogen			
Taken up by crops	40	47	64	80	96	80	103
Supplied							
with farmyard manure	7	7	8	9	8	6	6
with fertilizer	–	16	57	79	96	55	23
with by-products	–	–	6	8	12	10	14
Total supplied	7	23	71	96	116	71	43
Balance	–33	–24	7	16	20	–9	–60
Intensity of balance, %[1]	18	49	111	120	121	89	42
				Phosphorus (P_2O_5)			
Taken up by crops	15	18	24	29	38	33	42
Supplied							
with farmyard manure	7	8	9	8	6	6	
with fertilizer	–	12	37	63	66	20	4
with by-products	–	–	3	4	3	3	4
Total supplied	7	19	48	76	77	29	14
Balance	–8	1	24	47	39	–4	–28
Intensity of balance, %[1]	4	106	200	262	201	88	30
				Potassium (K_2O)			
Taken up by crops	38	48	61	76	84	71	88
Supplied							
with farmyard manure	16	18	20	21	15	12	12
with fertilizer	–	7	45	82	71	29	6
with by-products	–	–	17	25	24	18	26
Total supplied	16	25	82	128	110	59	44
Balance	–22	–23	21	52	26	–12	–44
Intensity of balance, %[1]	42	52	134	168	131	84	49

[1]Quotient expressing how much nutrient (as a percentage) taken up by the yield was replaced in all

Environmental studies

Nitrogen is widely used in agricultural practice in different organic and inorganic forms to enhance crop productivity. After the growing season, part of the nitrogen remains in forms sensitive to changes in conditions, such as nitrate. In years with above-average precipitation a significant amount of nitrate may leave the rooting zone of various crops even when land is cropped annually. The integration of knowledge related to the environmental conditions of a certain area with the soil, water and crop management practices helps to prevent the simultaneity of unfavourable processes leading to nitrate leaching, thus water resources may be protected from nitrate pollution of agricultural origin. It is of increasing importance that such an approach be applied in Hungarian crop production. Since the great spatial variability of soil-forming factors is clearly reflected by the heterogeneous (sometimes mosaic-like) soil cover in Hungary, the differentiation of categories within the soil types is essential in agricultural fertilizer practices, as reflected in the environment-friendly fertilizer recommendation system introduced in recent years.

A comparison of the results of several long-term field trials, conducted simultaneously at several experimental sites differing in environmental characteristics such as soil type and climatic conditions, provided a good basis for a more generalized quantification of the overall turnover of nitrogen and for calculating improved N balances. The results indicated that when the rational use of organic manures and N-containing fertilizers is based on the plant's N demand, the nitrogen balances can be kept in equilibrium. The amount of N needed to obtain economically viable yields, while at the same time being in balance with the requirements of the crops and keeping the environment uncontaminated, varied in long-term trials between 0 to 50 kg/ha/yr on fertile soils, 50 to 100 kg/ha/yr on coarse-textured soils, and 100 to 150 kg/ha/yr in farm field analysis. The residual effects of nitrate in long-term experiments proved that after the proper application of N fertilizer the amount of residual nitrogen was low and no nitrate-N accumulation was detected under the root zone even for coarse-textured soils (Németh, 1995).

The fate and behaviour of the surplus nitrogen in the soil-plant system was studied in several long-term field experiments (Németh, 1996, a, b, c). In one, on a calcareous chernozem soil, the mineral-N content of the upper 60 cm soil layer and the seasonal dynamics of the N forms were investigated after the application of four different N fertilization rates over an eleven-year period and after 12 years. The movement, accumulation and distribution of the NO_3-N in profiles down to six metres after 12 years is illustrated in Figure 1 (Németh, 1996b, c; Németh et al., 1987–88). Significant nitrate-N accumulation was found between 60 and 200 cm in the intensively fertilized treatments (200 and 300 kg N/ha/year). There was practically no difference in the nitrate-N distribution in unfertilized plots and in those which received the lower application rate (100 kg N/ha/year). The amounts of nitrogen accumulated in the profiles of plots fertilized yearly with 300 kg N/ha were ten times higher than those measured in the unfertilized plots. The effect of overfertilization on the residual nitrate-N form could be detected down to 350–400 cm (Németh et al., 1987–88).

The nitrogen (mostly nitrate) entering the deeper soil layers and/or the groundwater originates from various sources, one of them being the non-point load from agricultural land use. The contamination of shallow groundwater is thus partly caused by intensive agricultural activities, via increased nitrate leaching.

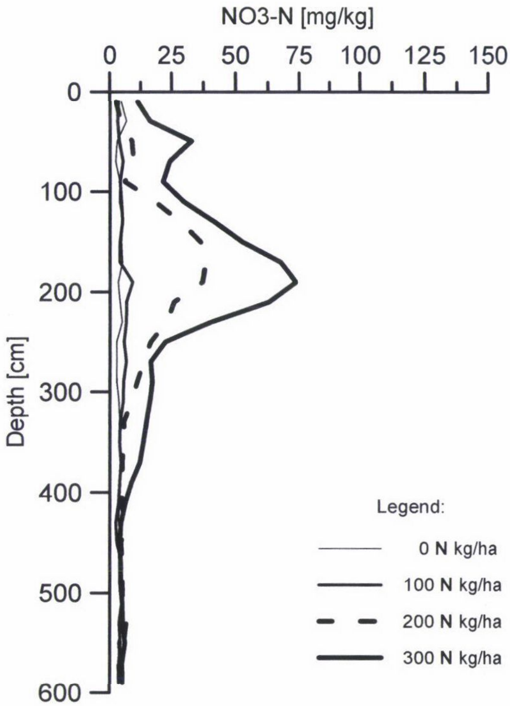

Fig. 1. Nitrate-N distribution in soil profiles of a 12-year field experiment

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Review

ROLE OF SOIL MULTIFUNCTIONALITY IN FUTURE SUSTAINABLE AGRICULTURAL DEVELOPMENT

G. VÁRALLYAY

RESEARCH INSTITUTE FOR SOIL SCIENCE AND AGRICULTURAL CHEMISTRY (RISSAC) OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY

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Soils represent a considerable part of the natural resources of Hungary. Consequently, rational land use and proper soil management – to guarantee normal soil functions – are important elements of sustainable (agricultural) development, having special importance both in the national economy and in environment protection. The main soil functions in the biosphere are as follows:

- conditionally renewable natural resource;
- reactor, transformer and integrator of the combined influences of other natural resources (solar radiation, atmosphere, surface and subsurface waters, biological resources), site of "sphere interactions";
- medium for biomass production, primary food-source of the biosphere;
- storage of heat, water, plant nutrients and – in some special cases – wastes;
- high capacity buffer medium, which may prevent or moderate the unfavourable consequences of various environmental stresses;
- natural filter and detoxication system, which may protect the deeper geological formations and the subsurface waters from various pollutants;
- significant gene reservoir, an important element of biodiversity;
- conservator of natural and human heritages.

Society has utilized these functions in different ways (rate, method, efficiency) throughout history, depending on the given natural conditions and socio-economic circumstances. In many cases the character of the particular functions has not been properly taken into consideration during the utilization of soil resources, and misguided management has resulted in their over-exploitation, in the decreasing efficiency of one or more soil functions, and – above a certain limit – in serious environmental deterioration.

The scientifically based planning and implementation of sustainable land use and rational soil management to ensure desirable soil functions, without any undesirable environmental side-effects, require the efficient control of soil processes.

Key words: soil functions, sustainable land use, control of soil processes, environmental stresses, degradation, pollution, soil moisture control

Introduction

Each society wishes to create favourable living conditions for its members. "Life quality criteria" are formulated in different ways by various societies or individuals, depending on the given geographical and socio-economic conditions, living standards, national, ethnical and religious traditions, history, policy, age, sex, educational level, position in the social hierarchy, etc. However, there is full agreement on the need for three elements:

- healthy, good quality food, and food security;
- clean water;
- a pleasant environment.

All three are closely related to rational land use and the sustainable management of land resources (Várallyay, 2002b).

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Sustainable development

"Sustainable development" is a global objective which needs to be realized everywhere and in all fields, i.e. every region or country, whether it is advanced or developing, and in each branch of the economy, ranging from settlement policy to agriculture, from health care to industry or infrastructure development (Láng, 1995; Proc. of Seminar on Technologies...., 1985; Várallyay and Németh, 1996; Várallyay, 2000d; Greenland and Szabolcs, 1993).

The term "sustainable development" was not yet mentioned at the UN Conference on the Human Environment (Stockholm, 1972). Twenty years later it became the most fashionable and most frequently used term at the World Summit on the Environment (Rio de Janeiro, 1992), and it was the title of the Rio+10 Conference on Sustainable Development (Johannesburg, 2002).

The two parts of the expression ("sustainable" and "development") could be a potential point of conflict in themselves. They have been interpreted (and translated) in different ways in various countries, depending on their environmental and socio-economic conditions, historical background and even political situation, with more emphasis being laid sometimes on "development" and sometimes on "sustainability". Consequently, the realization of the declared aims and formulated objectives requires global compromises and joint efforts instead of regional or national economic and/or social confrontation.

FAO formulated the following definition in 1988: "Sustainable development is the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development (in agriculture, forestry and fisheries sectors) conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable." The definition formulated jointly by IUCN, UNEP and WWF tends to emphasize the biological elements: "Sustainable development is improving the quality of human life while living within the carrying capacity of supporting ecosystems" (Várallyay, 1997a,d).

The basic concept was adopted in Hungary according to the country's natural endowments and socio-economic circumstances in the "AGRO-21" Programme (1995).

Sustainable development includes efficient multifunctional agriculture (using environment-friendly, energy- and material-saving technologies and paying special attention to quality) and socially acceptable rural development, simultaneously. The given land resources (geological formations, relief, atmosphere, surface and subsurface water resources, soil biota, vegetation) are used, managed and – hopefully – protected by society according to its requirements, depending on the given socio-economic conditions, modified by the historical background and formulated by decision-makers at various levels (Fig. 1).

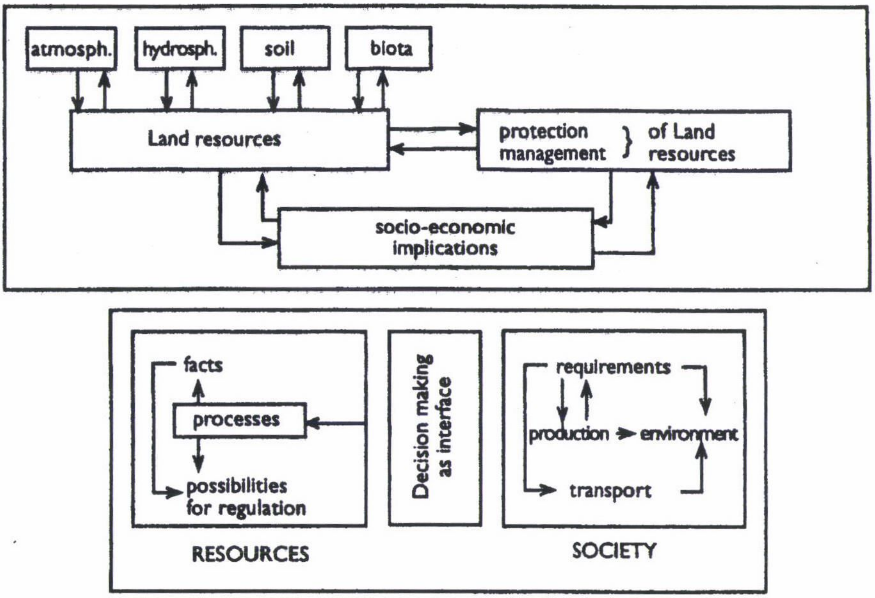

Fig. 1. Relationships between resources and society

Soil resources and soil functions

Land (the soil–water–near the surface atmosphere continuum, with its geology, relief and biota) represents a considerable part of the natural resources of Hungary (Láng et al., 1983; National Atlas of Hungary, 1989; Várallyay et al., 1985; Várallyay, 1998b, 1999, 2000a). Consequently, rational land use and proper soil management – to guarantee normal soil functions – are important elements of sustainable (agricultural) development, having special importance both in the national economy and in environment protection.

In our present world, soil is much more than the most important medium for primary biomass production. Consequently, it has to be managed as a multifunctional natural resource and its other functions have to be taken into consideration in the mechanism for optimizing rational land use. The main soil functions and their role in the biosphere and in the biogeochemical cycles of natural or human-applied elements or compounds can be summarized as follows (Várallyay, 1997c, 1998b):

• Soils are the most significant – conditionally renewable – natural resources. During rational biomass production they do not change irreversibly, their quality does not decrease unavoidably and fundamentally, but their "renewal", based on soil resilience does not happen automatically: soil conservation, the maintenance and increase of soil fertility, requires permanent activities, such as sustainable land use, agronomic measures, remediation and reclamation.

• Soil is a reactor and transformer, integrating the combined influences of other natural resources, such as solar radiation, atmosphere, surface and subsurface waters, deeper geological strata and biological resources. Their biogeochemical cycles develop a "life medium" for microbiological activities, as well as an ecological environment (landsite) for natural vegetation and cultivated crops. The pedosphere forms under the integrated influence of the lithosphere (geology, parent material), the atmosphere (climate, weather), the hydrosphere (surface and subsurface water resources), and the biosphere, but – at the same time – the pedosphere influences and modifies the mass and energy regimes of these spheres, as demonstrated in Figure 2.

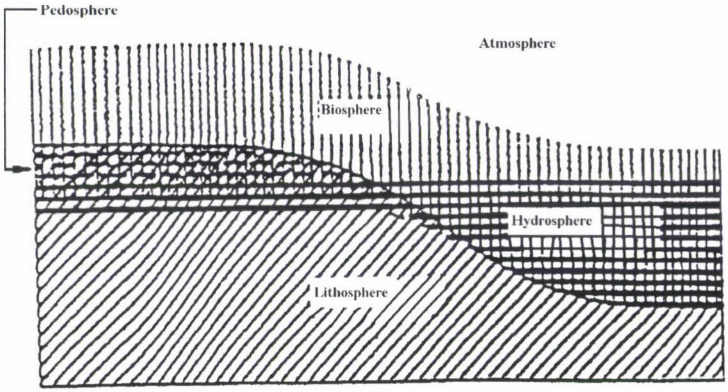

Fig. 2. The pedosphere

• Soil is the most important medium for biomass production (food, fodder, raw material for industry, alternative energy). Soil, as a four-dimensional [spatial (horizontal and vertical) variability and temporal dynamism] three- (or four-) phase, polydisperse system can simultaneously satisfy – to a certain extent – the ecological requirements (air, water and nutrient supply) of living organisms, the natural vegetation and cultivated crops. This special ability is the unique soil property: soil fertility, which varies greatly and has changed considerably depending on natural factors and human activities, such as land use and soil management. The territorial distribution of the most important soil characteristics determining their agro-ecological potential (soil fertility, soil productivity) is summarized in Table 1 (Láng and Csete, 1992; Láng et al., 1983; Várallyay et al., 1985). Soil is the primary food source of the biosphere, the starting point of the food chain.

• Soil is a major natural store of heat, water, plant nutrients and other elements, including wastes and (potentially) harmful elements or chemical compounds. The stored water and plant nutrients ensure the continuous moisture and nutrient supply of plants – according to their uptake dynamics – for shorter or longer periods without any additional supply (rain, irrigation, nutrient application). This soil function is the basis of rational moisture regime control and plant nutrition (Várallyay et al., 1985, 1992; Várallyay, 1994a, 1997c, 2002a).

• Soils represent a high capacity buffer medium for the biosphere, which, within certain limits, may moderate the various stresses caused by environmental factors (climatic droughts or excessively humid conditions, frost, etc.) and/or human activities (high input, fully-mechanized and chemically controlled crop production; liquid manure from large-scale livestock farms; wastes and waste waters originating from industry, transport, urban and rural development, etc.). Buffer systems have strict limits and boundary conditions. Sometimes this is forgotten by the "users", which leads to serious environmental problems. To prevent and avoid unfavourable side-effects, the tolerance limits must be identified, quantified, precisely determined and clearly formulated. This can only be done on the basis of comprehensive sensitivity (susceptibility, vulnerability) studies and impact analyses. Intensive international, regional and national studies have been carried out to determine these tolerance limits and target conditions. In Hungary comprehensive studies have identified and sometimes quantified the susceptibility of soils to wind and water erosion, acidification, salinization/alkalization/sodification, physical soil degradation (compaction, structure destruction, surface sealing) and chemical pollution. As an example, a simplified map of the susceptibility of soils to acidification is shown in Figure 3 (Várallyay et al., 1993; Várallyay, 2000b, d).

• Soil is an efficient "natural filter" and detoxication system that may prevent the deeper horizons and the subsurface waters from becoming contaminated by various pollutants deposited on the soil surface or put into the soil. A good example of such an assessment is the map of the nitrate leaching hazard, presented in Figure 4, according to Németh and his research team.

• Soil is a significant gene reservoir for the biosphere and thus an important element in biodiversity. A considerable proportion of living organisms live in or on the soil or are closely related to (sometimes depending on) the soil. This function has particular significance in the stabilization and conservation of biodiversity.

• Soil is the conservator of natural and human heritages.

These functions are all equally important, but society has used them in different ways (rate, method, efficiency) throughout history, depending on the given natural conditions and socio-economic circumstances. In many cases the character (territorial and temporal variabilities, changeability–stability–controllability, boundary conditions, limitations) of a certain function was not (properly or adequately) taken into consideration during the utilization of soil resources. In such cases the misguided management resulted in over-exploitation, decreasing the efficiency of one or more soil functions, and – above a certain limit – causing serious environmental deterioration (Várallyay, 1997c, 2002b).

Table 1

Territorial distribution of the various factors determining the agro-ecological potential in Hungary (in hectares)

Soil factors determining the agro-ecological potential	Total	%
PARENT MATERIAL		
1. Glacial and alluvial deposits	3 433 430	37.7
2. Loess, loess-like deposits	4 373 920	48.0
3. Tertiary and older deposits	681 440	7.5
4. Volcanic clayey tuffs ("Nyirok")	151 660	1.7
5. Limestone, dolomite	238 950	2.6
6. Sandstone	11 430	0.1
7. Shale, phyllite	38 530	0.3
8. Granite, porphyrite	9 740	0.1
9. Andesite, riolite, basalt	179 350	2.0
SOIL REACTION AND CARBONATE STATUS		
1. Strongly acidic soils	1 228 930	13.5
2. Slightly acidic soils	3 848 550	42.2
3. Calcareous soils (effervescence with dilute acid from the surface)	3 496 090	38.4
4. Salt-affected soils, calcareous from the surface	385 260	4.2
5. Salt-affected soils, non-calcareous from the surface	153 620	1.7
SOIL TEXTURE		
1. Sand	1 437 230	15.8
2. Sandy loam	875 460	9.6
3. Loam	3 932 320	43.2
4. Clay loam	1 692 630	18.6
5. Clay	632 840	6.9
6. Organic soils (peat, partly decomposed peat)	117 560	1.3
7. Coarse fragments (gravel, non- or partly weathered rocks, etc.)	421 410	4.6
SOIL WATER MANAGEMENT PROPERTIES		
1. Soils with very high infiltration rate (IR), permeability (P) and hydraulic conductivity (HC), low field capacity (FC) and very poor water retention (WR)	957 420	10.5
2. Soils with high IR, P and HC, medium FC and poor WR	1 009 910	11.1
3. Soils with good IR, P and HC, good FC and good WR	2 264 230	24.9
4. Soils with moderate IR, P and HC, high FC and good WR	1 735 640	19.1
5. Soils with moderate IR, poor P and HC, high FC and high WR	571 080	6.2
6. Soils with unfavourable water management, low IR, very low P and HC and high WR	1 349 750	14.9
7. Soils with extremely unfavourable water management, very low IR, extremely low P and HC, and very high WR	329 210	3.6
8. Soils with good IR, P and HC, and very high FC	117 560	1.3
9. Soils with extreme moisture regime due to shallow depth	774 650	8.4
ORGANIC MATTER CONTENT (t/ha)		
1. 0–50	481 750	5.3
2. 50–100	1 915 130	21.0
3. 100–200	2 586 270	28.5
4. 200–300	1 923 590	21.1
5. 300–400	1 887 270	20.7
6. 400–	305 440	3.4
DEPTH OF THE SOIL (limited by solid or slightly fragmented rocks, gravel, cemented layers, pans, peat, loose sand, groundwater, etc.)		
1. 0–20 cm	25 780	0.3
2. 20–40 cm	445 260	4.9
3. 40–70 cm	480 310	5.3
4. 70–100 cm	370 630	4.0
5. 100– cm	7 787 470	85.5

Fig. 3. Map of the susceptibility of Hungarian soils to acidification. 1. Strongly acidic soils (13.5%). 2. Highly susceptible soils due to their low buffering capacity (slightly acidic soils with light texture and low organic matter content) (14%). 3. Susceptible soils due to their medium buffering capacity (slightly acidic soils with medium texture and organic matter content) (5.0%)..4. Moderately susceptible soils due to their high buffering capacity (slightly acidic soils with heavy texture and/or high organic matter content) (23.2%). 5. Slightly susceptible soils (salt-affected) (5.9%). 6. Non-susceptible soils (calcareous from the surface) (38.4%)

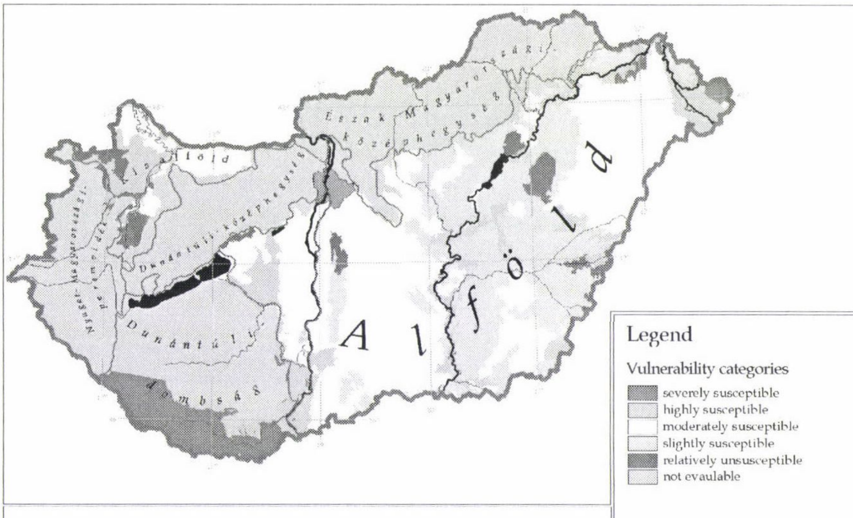

Fig. 4. Vulnerability of soils to N-leaching in Hungary

Soil processes and their control

The multifunctionality of the soil is determined by the combined influences of soil properties, which are the results of soil processes (mass and energy regimes, abiotic and biotic transport and transformation, and their interactions) under the combined influences of soil-forming factors. Any soil-related human activity influences the soil through these processes. Consequently, the control of soil processes is a great challenge and the main task of soil science and soil management is sustainable development (Várallyay, 1994b, 1997b, 2000b, c).

The conceptual model of a rational strategy for the control of soil processes is illustrated in Figure 5.

In spite of the fact that Hungarian natural conditions are relatively favourable for rainfed biomass production, more than half the soils are subject to various ecological constraints (Szabolcs and Várallyay, 1978; Várallyay, 1993b, 1999) (Fig. 6) and unfavourable soil processes:

• Soil degradation processes. The main soil degradation processes are: soil erosion by water or wind; soil acidification; salinization and/or alkalization; physical degradation (structure destruction, compaction); extreme moisture regime: drought sensitivity and waterlogging hazard; biological degradation; unfavourable changes in the plant nutrient regime; decrease in natural buffering capacity, soil (and water) pollution (Szabó et al., 1998; Várallyay, 1989a, 1998b, 2000b).

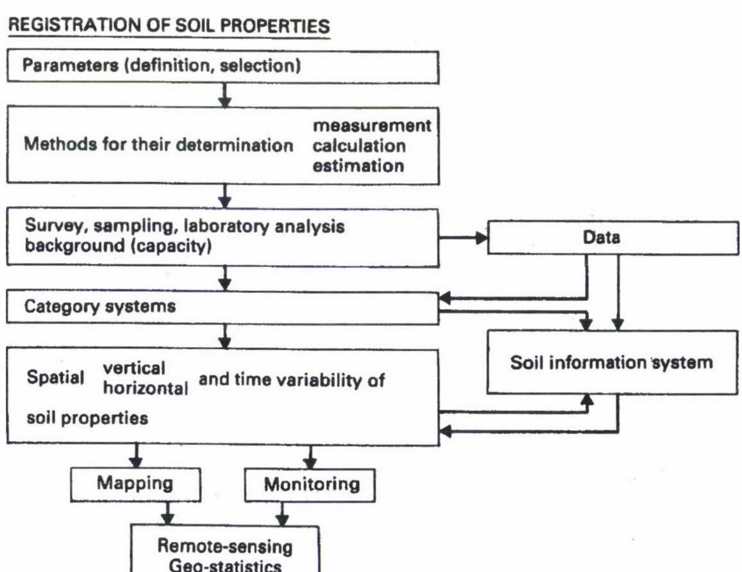

Fig. 5. Control of soil processes

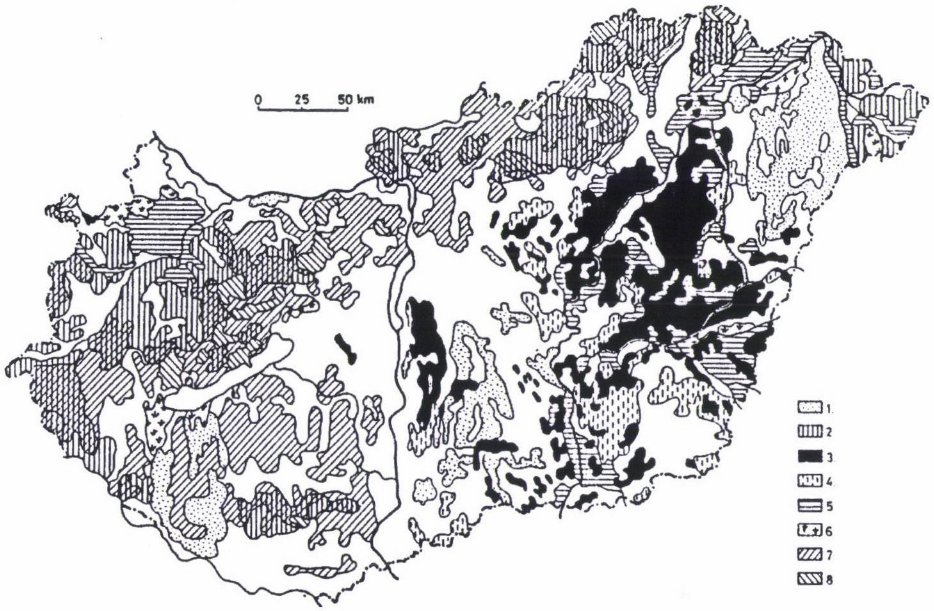

Fig. 6. Map of the limiting factors of soil fertility in Hungary (original scale: 1:500,000). 1. Extremely coarse texture. 2. Acidity. 3. Salinity and/or alkalinity. 4. Salinity and/or alkalinity in the deeper layers. 5. Extremely heavy texture. 6. Waterlogging. 7. Erosion. 8. Shallow depth

• Extreme moisture regime. The annual precipitation (especially on the Hungarian plains) shows extremely high spatial and temporal variability – even on a micro-scale. Non-uniform rainfall distribution, the heterogeneous microrelief of the "flat" Hungarian Plain, and the unfavourable hydrophysical properties of soils are the main reasons for the extreme moisture regime: the simultaneous hazard of waterlogging or over-moistening and drought sensitivity on large areas, sometimes in the same places within a short period. Based on a detailed assessment of the physical and hydrophysical properties of soils (texture, structure; water retention curves: total and field capacity, wilting percentage, available moisture range; infiltration rate; saturated and unsaturated hydraulic conductivity) and on the factorial analysis of field water balance and soil moisture regime the general conclusion was drawn that 43% of Hungarian soils can be characterized by unfavourable, 26% by moderate and 31% by good moisture regime. Their extent is illustrated in Figure 7 for the whole country, and in Figure 8 for the administrative regions of Hungary, indicating the main reasons for the differences (Várallyay, 1989b, 1997b, 2001).

• Nutrient stresses. The deficiency or accumulation and/or toxicity of one or more elements in the biogeochemical cycle are strongly increasing environmental threats, mainly due to the non-scientifically based, inadequately controlled, sometimes over-chemized social development, including biomass production and waste management (Kádár, 1991; Várallyay, 1994a, 2002a; Várallyay and Németh, 1996; Várallyay et al., 1992).

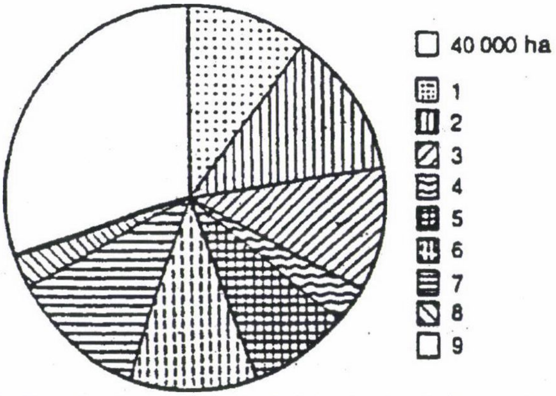

Fig. 7. Distribution of soils according to their hydrophysical properties in Hungary.
1–5 = Soils with unfavourable hydrophysical properties: 1: due to very coarse texture; 2: due to very heavy texture; 3: due to strong salinity–alkalinity; 4: due to waterlogging; 5: due to shallow depth; 6–8 = Soils with moderately unfavourable hydrophysical properties: 6: due to coarse texture; 7: due to heavy texture or clay accumulation in the B horizon; 8: due to moderate salinity/alkalinity in the deeper layers; 9 = Soils with good hydrophysical properties

• Environmental pollution. The accumulation or mobilization of various, potentially harmful (or even toxic) elements (or compounds) in the "life media" of various organisms, in air, in water, in soil; or in the biomass of various organisms within the soil–water–plants–animals–human beings "food chain" (Kádár, 1991; Várallyay, 1993a, 1996, 1998a, b).

The scientifically based planning and implementation of sustainable land use and rational soil management require adequate soil information: exact, reliable, "detectable" (preferably measurable) and accurate, quantitative territorial data on well-defined soil and land properties, including the characterization of their spatial (vertical, horizontal) and temporal variabilities and pedotransfer functions; on the soil processes and biogeochemical cycles, including their determining and influencing factors and their mechanisms, and on the actual and/or potential impacts of human activities.

In Hungary a large amount of information is available on the ecological conditions, including soils, as a result of long-term observations, and various soil surveys, analytical and mapping activities conducted at the national (1:500,000), regional (1:100,000), farm (1:10,000–1:25,000) and field levels (1:5,000–1:10,000) over the past 60 years (Fésűs et al., 2000; Várallyay, 1993a, 1994c). Recently a considerable part of these data have been organized into computer-based GIS databases giving opportunities for the prevention, elimination or reduction of environmental stresses and their unfavourable consequences (Szabó et al., 1998; Várallyay, 1994c).

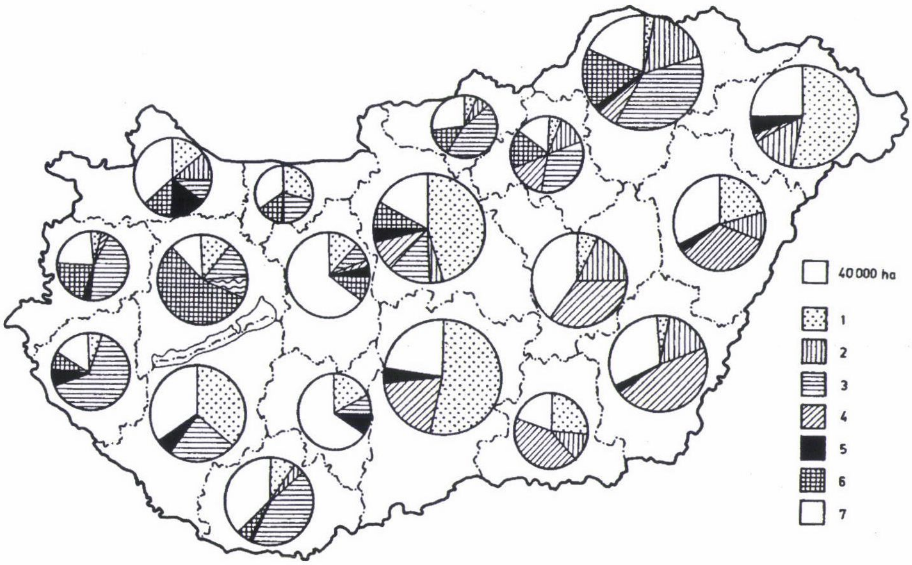

Fig. 8. Distribution of soils according to their hydrophysical properties in the counties (administrative districts) of Hungary. 1–6: Soils with unfavourable hydrophysical properties: 1: due to coarse texture; 2. due to heavy texture; 3: due to clay accumulation in the B horizon; 4: due to salinity/alkalinity; 5: due to waterlogging; 6: due to shallow depth. 7 = Soils with good hydrophysical properties

Main elements of rational land use and soil management for sustainable, multifunctional agriculture

Efficient, economically viable, socially acceptable and environmentally sound sustainable land management includes the following main elements (Várallyay, 1994b, 1997a; Várallyay and Németh, 1996):

1. Territorial coordination of the agroecological conditions (land-site characteristics) and the agro-ecological requirements of cultivated crops, taking into consideration both production and environmental aspects on short-, mid- and long-term scales:
   - rational land use and optimization of cropping patterns under the given (and hardly controllable) natural conditions;
   - selection of proper crops (crop rotations) and cultivars for the given natural circumstances (selection and breeding of varieties or genotypes tolerant of certain ecological constraints, such as frost, soil acidity, salinity/alkalinity, drought, moisture surplus, extreme soil texture, soil compaction, etc.);
   - improvement of (soil) ecological conditions according to crop requirements (amelioration of land, reclamation/improvement of soil, soil conservation, irrigation and drainage, proper agronomic measures).

2. Rationalization of the structure of agricultural fields (under the new ownership structure):

- optimization of field size according to the given physiographic conditions: development of more homogeneous fields for uniform agrotechnical measures, taking into consideration a rational level of biodiversity;
- development of proper infrastructure: rational territorial arrangement of fields, roads, canals, buildings, forest shelter belts, etc., taking into consideration landscape preservation requirements, as well.

3. Reduction (minimalization) of production wastes: plant residues; animal excrements, such as organic manure and liquid manure; wastes from yield and food processing, with their most efficient recycling without any harmful environmental side-effects.

4. Control (prevention, elimination or at least moderation) of undesirable soil degradation processes. Priority must be given to efficient preventive measures. For successful prevention satisfactory prognosis is necessary. This can be rationally based on a comprehensive
- sensitivity analysis: evaluating the susceptibility of soils to various soil degradation processes;
- impact analysis: evaluation and forecast of the potential "positive" and "negative" impacts of various human activities.

5. Improvement of the efficiency of agricultural water management and soil moisture control. The greatly variable climate and weather conditions, the relief (undulating surface and the heterogeneous microrelief of the "flat" Hungarian Plain), and the unfavourable hydrophysical properties of some soils necessitate special "two-way" soil moisture control. Because both irrigation and drainage have serious ecological and economic limitations every effort should be made
- to increase water storage within the soil in plant-available form without any unfavourable environmental consequences: to help infiltration into the soil; to increase the water storage capacity; to reduce the immobile and not plant-available moisture content;
- to reduce evaporation, surface runoff and filtration losses of water (atmospheric precipitation and irrigation water);
- to improve the vertical and horizontal drainage conditions of the soil profile or the given area (prevention of over-saturation and waterlogging).

Most of these measures are – at the same time – an integral part of environment protection (Table 2).

6. Precision plant nutrient management:
- rational use of fertilizers based on crop requirements (dynamics of their nutrient uptake), soil conditions and agroclimate;
- efficient recycling of crop residues and production wastes (including organic manure);
- utilization of other wastes with utilizable plant nutrient content and without potentially harmful chemical compounds.

7. Soil pollution control. The main elements of efficient soil pollution control – based on a comprehensive assessment of the quantity, status and regime of pollutants in the soil–water–plant–near the surface atmosphere continuum and in the food-chain (Fig. 9), and on the evaluation of their ecological impacts and environmental hazards – are as follows:

- emission/imission reduction (preventing or reducing the quantity of pollutants deposited on or transported to the soil surface or into the soil);
- prevention of the mobilization of potentially harmful chemical compounds or elements which are already present in the soil but in – temporarily – immobile form;
- decrease in the susceptibility/vulnerability of soil to various pollutants (through an increase in the buffering capacity of soils), making it tolerant of a higher critical load of pollutants and consequently reducing the "exceedance risk" and the unfavourable ecological consequences.

The conceptual model of such a system is shown in Figure 10.

Table 2

Elements and methods of soil moisture control with their environmental impacts (EI)

Elements	Methods	EI*
Reducing		
surface runoff	Increase in the duration of infiltration (moderation of slopes; terracing contour ploughing; establishment of permanent and dense vegetation cover; tillage; improvement of infiltration; soil conservation farming system)	1,1a,5a,8
evaporation	Helping infiltration (tillage, deep loosening); Prevention of runoff and seepage, water accumulation	2,4
feeding of ground-water by filtration losses	Increase in the water storage capacity of soil; moderation of cracking (soil reclamation); surface and subsurface water regulation	5b,7
rise of the water table	Minimalization of filtration losses (↑); groundwater regulation (horizontal drainage)	2,3,5b,5c
Increasing		
infiltration	Minimalization of surface runoff (tillage practices, deep loosening) (↑)	1,4,5a,7
water storage in soil in available form	Increase in the water retention of soil; adequate cropping pattern (crop selection)	4,5b,7
Irrigation	Irrigation; groundwater table regulation	4,5c,7, 9,10
Surface	surface	1,2,3,5c,6,7, 11
drainage	moisture control (drainage)	
Subsurface	subsurface	

* Reference numbers: see below

Favourable environmental effects

Prevention, elimination, limitation or moderation of: water erosion (1); sedimentation (1a)
secondary salinization, alkalization (2)
peat formation, waterlogging, overmoistening (3)
drought sensitivity, cracking (4)
plant nutrient losses by:
surface runoff (→ surface waters eutrophication) (5a)
leaching (→ subsurface waters) (5b); immobilization (5c)
formation of phytotoxic compounds (6)
"biological degradation" (7)
flood hazard (8)

Unfavourable environmental effects

overmoistening, waterlogging, peat and swamp formation, secondary salinization/alkalization (9)
leaching of plant nutrients (10)
drought sensitivity (11)

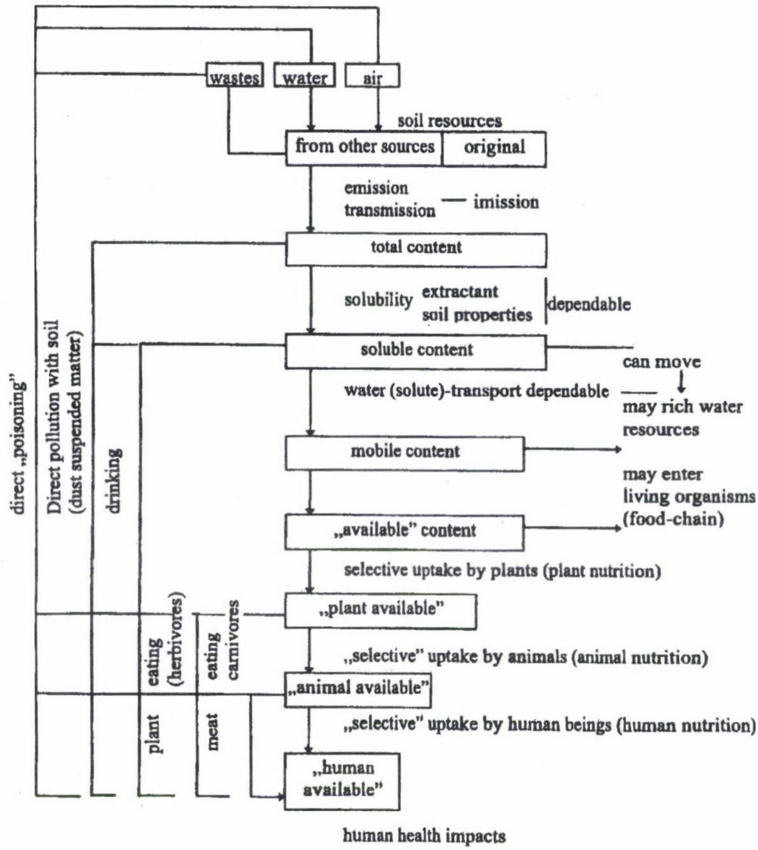

Fig. 9. Sources and pathways of soil pollution

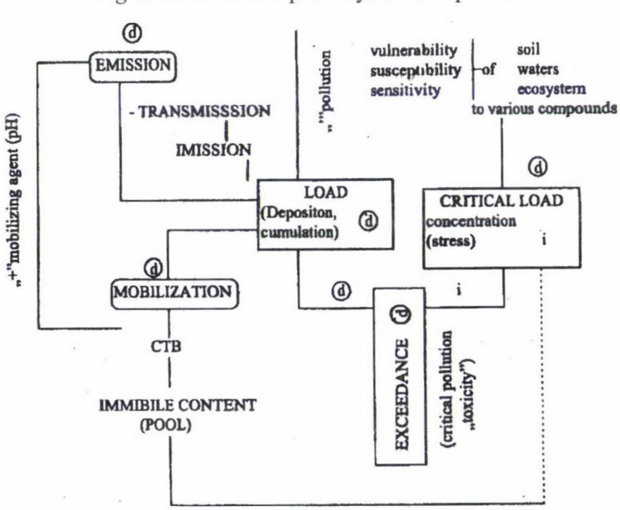

Fig. 10. Strategy for pollution control (i: increase; d: decrease)

Conclusions

Agricultural production is economy driven. In contrast, the maintenance of soil multifunctionality, the quality of surface and subsurface water resources and the protection of the natural environment are not (fully) economy-dependent elements of sustainable development, but imperative tasks of human society. All actions which aim at or assist soil multifunctionality are important elements in sustainable multifunctional agricultural development. These are tasks jointly facing the state, decision makers at various levels, land owners, land users, and – to a certain extent – each member of society. The joint efforts of all will be required to harmonize agricultural production and environment protection to give an improvement in the quality of life.

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Review

HISTORY OF ACTA AGRONOMICA HUNGARICA

J. SUTKA

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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Hungarian agricultural scientists who published new research results in the 1950s generally submitted their manuscripts to Acta Agronomica Hungarica, which also provided a forum for the development of international cooperation.

When the journal was established it published original papers, reviews, lectures and short communications on agricultural sciences in English, Russian, German and French. It was edited in Budapest, first by András Somos and later by János Surányi. In 1965 the editorial office was transferred to the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, where Sándor Rajki converted it into an English language journal and also made substantial changes to its structure.

From 1983 Acta Agronomica was edited in the University of Horticulture and Food Industry, Budapest, with István Tamássy and later Pál Kozma as chief editor. After 12 years, in May 1995, the Agricultural Sciences Section of the Hungarian Academy of Sciences again charged the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, with the editing of the journal, and since 2000 Zoltán Bedő has been the chief editor.

The editorial board of Acta Agronomica Hungarica still regards the publication of the results achieved in basic and applied research on agricultural science as its primary task, with the emphasis on crop research. Preference is given to research on physiology, genetics, crop production, plant breeding, cell and molecular biology, nature and environment protection, and the preservation of gene reserves.

The professional standard, recognition, market value and time to publication have improved considerably in recent years. This can be attributed partly to the setting up of an International Advisory Board in addition to the Hungarian Editorial Committee, and partly to the computerised editing and to the precise, conscientious work of the reviewers.

Key words: Acta Agronomica Hungarica, history, Editorial Board, author, scientific paper, scientific field

Introduction

The reorganisation of the research network of the Hungarian Academy of Sciences in the early fifties opened up a new era in Hungarian agricultural research. Both at the domestic and international level there was great need for a forum where the research results achieved in this field could be published. A further aim was to follow international research trends and results and, last but not least, to establish international scientific cooperation. In 1950 the Hungarian Academy of Sciences thus decided to set up a multidisciplinary journal, edited in Budapest, under the name Acta Agronomica Hungarica.

Acta Agronomica Hungarica is published in one volume of four issues each year. An exception to this was made in two of the fifty volumes published so far: Volume 11 covered two years, 1961 and 1962, as did Volume 43 (1994–1995).

The history of Acta Agronomica Hungarica can be divided into four periods, according to the location of the editorial office:

Period I	1950–1964	Volumes 1–13
Period II	1965–1982	Volumes 14–31
Period III	1983–1995	Volumes 32–43
Period IV	1995–2002	Volumes 44–50

Period I

During the first period the journal was edited in Budapest by András Somos, and later by János Surányi. The members of the Editorial Committee in the year the journal was founded are listed in Table 1. Papers were published in English, French, German and Russian. The first 13 volumes included a total of 190 papers, 166 by Hungarian authors and 24 by foreign scientists.

Table 1
Composition of the Editorial Committee in various years

1950	1980	1993	2002
	Chief Editor: S., Rajki	*Chief Editor:* I., Tamássy	*Chief Editor:* Z., Bedő
Editor: A., Somos	*Editor:* Gy., Pál .	*Editor:* Máthé Á.	*Editor:* Sutka J.
Members:	*Members:*	*Members:*	*Members:*
Z., Fekete	A., Horn	S., Rajki	E., Balázs
B., Győrffy	P., Kozma	I., Dimény	E., Bocz
A., Horn	G., Láng	B., Győrffy	I., Dimény
I., Oszkályi	G. A., Manninger	A., Horn	J., Dohy
K., Páter	I., Máthé	Z., Király	P., Kozma
I., Rázsó	I., Szabolcs	P., Kozma	E., Kurnik
K., Sedlmayr	I., Tamássy	E., Kurnik	I., Láng
G., Ubrizsi		I., Láng	Gy., Várallyay
I., Vágsellyei		I., Máthé	
		I., Szabolcs	

Period II

In 1965 the Editorial Committee of Acta Agronomica Hungarica was transferred to the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár, where the new Editor-in-Chief, Sándor Rajki, and Editor, Gyula Pál, decided that instead of being published in four languages, the journal should be written only in English, with Russian summaries. During the years when it was edited in Martonvásár the structure of the journal underwent substantial changes, as the result of which it deviated from international practice. Instead of original papers, much of the journal was taken up by columns such as

Varia, Forum, Chronica, Recensiones, etc. The majority of papers included in these columns were not clearly divided into the usual Introduction, Materials and Methods, Results and Discussion sections and the name and association of the author were found at the end of the paper. Figures and tables were sometimes included, which helped in the interpretation of the articles. By the 1970s the Varia and Forum columns sometimes made up as much as 80% of the journal. The number of papers given in Table 2 refers only to standard papers. During these years the Editorial Committee consisted of Artúr Horn, Pál Kozma, Géza Láng, G. Adolf Manninger, Imre Máthé, István Szabolcs and István Tamássy.

Table 2
Number of Hungarian and foreign papers from 1950 to 2002

Period	Hungarian	Foreign	Total
1950–1964	166	24	190
1965–1982	330	65	395
1983–1995	208	163	371
1996–2002	142	199	341
Total	846	451	1297

Period III

In 1983 the Editorial Office of Acta Agronomica Hungarica moved again, this time to the University of Horticulture and Food Industry in Budapest, where it was edited for 12 years with the supervision of first István Tamássy and later Pál Kozma. The composition of the Editorial Committee during this period can be seen in Table 1. The Forum, Varia, etc. columns introduced in Period II were discontinued and the papers were grouped according to subject (Plant Physiology, Crop Production, Plant Genetics and Breeding, Zoophysiology, Reviews, Book reviews, etc.). It sometimes took as much as 2–3 years for submitted manuscripts to be published, and there were still more Hungarian than foreign papers in the journal (Fig. 1).

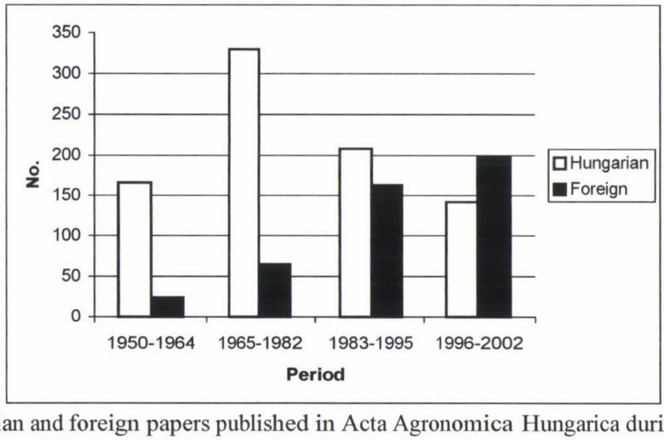

Fig. 1. Hungarian and foreign papers published in Acta Agronomica Hungarica during the various periods

Period IV

In May 1995 the Agricultural Sciences Section of the Hungarian Academy of Sciences again decided to transfer the Editorial Office of Acta Agronomica Hungarica from Budapest to the Agricultural Research Institute of the Academy in Martonvásár. To start with Pál Kozma continued to act as Editor-in-Chief, but he was succeeded in 2000 by Zoltán Bedő. The members of the Editorial Committee in 2002 are listed in Table 1. Four issues a year have been published regularly during the seven years since 1996.

While the professional profile of the journal has been narrowed to some extent, the Editorial Board of Acta Agronomica Hungarica still considers the publication of the results achieved in agricultural basic and applied research as its chief task. Preference is given to manuscripts dealing with cultivated crops and to those written by Hungarian scientists, but only if the topic of the paper is of current interest and the manuscript is of a high standard.

The professional standard of the journal and the manuscript processing time have considerably improved in recent years, and it has also achieved greater recognition. In order to give the journal greater prestige, an International Advisory Board was set up in 2000, the members of which also participate on occasion in reviewing the manuscripts. At present this advisory board has the following members: F. Altay (Turkey), E. P. Cunningham (Ireland), J. Glinski (Poland), I. Prášil (Czech Republic), M. Rousset (France), P. Smith (UK), P. Stamp (Switzerland) and A. M. Stanca (Italy).

All original papers consist of the following sections: Abstract, Key Words, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Manuscripts are now only accepted if they are written in English on a word processor and submitted by E-mail or on a floppy disc.

In the middle of the 1990s, when the Editorial Office first returned to Martonvásár, the manuscripts were typed, edited and page-set in the institute, but the rest of the work was done in the printing office. By a lucky chance, the printing office of the Academy Press is situated in Martonvásár, so it was easy to coordinate the work and make any necessary corrections within a short time. In this way, it was possible to reduce the time required to get through the press to 1–1½ months and we were able to keep to the deadline.

In 1999 the printing process was further modernised when digital printing was introduced. This means in practice that Acta Agronomica is printed using a large, complicated, high quality printer attached to a computer. Each issue is prepared in the form of a 'pdf' file and taken to the printer's on a compact disc. Digital printing not only results in better quality, but also means that the time required to get through the press has been further reduced to 2–3 weeks.

As digital printing requires all the manuscripts to be processed on the computer, this has also facilitated the inclusion of the journal on the internet. The homepage of the Academy Press can be found at: www.akkrt.hu. The Acta journals can be ordered in paper form or on-line (Table 3). If both forms are required, the subscription price is 20 % higher. Orders can be placed by E-mail at folyoirat@akkrt.hu. Individual subscribers can obtain a considerable reduction.

A comparison of the number of Hungarian and foreign original papers published over the 50 years shows clearly that in the 1950s and early 1960s there were substantially more Hungarian papers than foreign ones. This was particularly true during the second period. During the third period Hungarian manuscripts were still in the majority, but the difference was no longer very great. Since 1996 the trend has been reversed and far more manuscripts are now received from abroad than from Hungary (Fig. 1). The largest number of foreign papers are received from India, Egypt, Nigeria, etc.

All in all, over the four periods, Acta Agronomica has published a total of 1297 original papers, thus making a substantial contribution to making Hungarian research results known in many countries of the world.

Figure 2 shows the number of Hungarian and foreign papers published in the journal between 1996 and 2002.. With the exception of 1996 more manuscripts originated from abroad than from Hungary. The graph suggests that there may be a slight decline in the number of foreign papers and a slight rise in those from Hungary in 2003. Papers written as the result of research cooperation are also published in considerable numbers, but no significant trend can be observed in this connection.

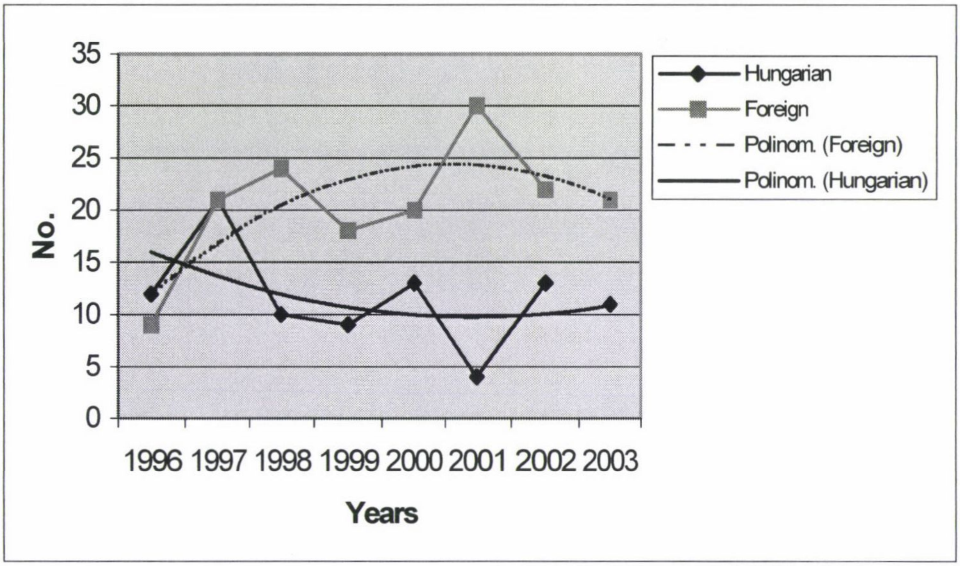

Fig. 2. Proportion of foreign and Hungarian papers in the years 1996–2002

A survey was also made of the ratio of papers published in various fields of agriculture over the seven years of the fourth period. The most papers were found to be on crop production (47%), plant genetics and breeding (20%) and plant physiology (16%), and the fewest on animal husbandry (3%) and plant protection (3%) (Fig. 3).

The funds available from the Publishing House of the Hungarian Academy of Sciences for the upkeep of the journal are very modest (Table 3), despite the fact that they have increased each year, now being double what they were seven years ago. It is thus impossible to pay any fees to either authors or reviewers. The Agricultural Research Institute of the Hungarian Academy of Sciences makes a regular financial contribution to the costs of publishing Acta Agronomica Hungarica.

Even in the mid-nineties the subscription price of Acta Agronomica Hungarica appeared to be rather high compared with foreign journals with a similar profile and size. Unfortunately, over the last seven years this price has increased by a further 43% (Table 3).

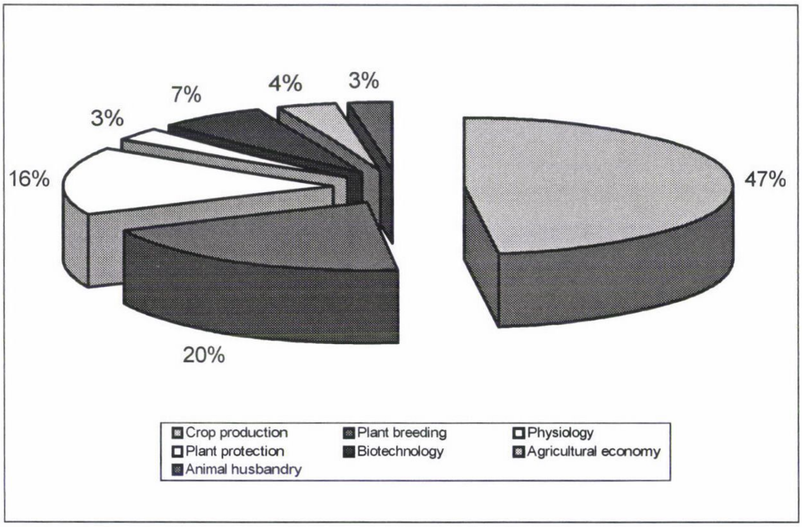

Fig. 3. Percentage distribution of papers according to fields of science over the last seven years

Table 3
Changes in the available funds and subscription prices between 1996 and 2002

Year	Volume	Funds (thousand Forints)	Subscription price (USD)
1996	44	120	112
1997	45	180	128
1998	46	200	156
1999	47	220	170
2000	48	240	196
2001	49	264	198
2002	50	284	198

Acknowledgements

Thanks are due to the Agricultural Sciences Section of the Hungarian Academy of Sciences for their moral and financial support, to the Hungarian and foreign scientists who supply us with papers in ever greater numbers and of improving standard, to the Editorial Committee and professional reviewers for their conscientious work, to the language editor, Barbara Harasztos, the typist, Magdolna Kocsis-Nagy and the technical editor, Béla Kőszegi, for their excellent cooperation, and to all those colleagues who have given their encouragement and support to the publication of the journal.

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Obituary

PROF. BÉLA GYŐRFFY (1928–2002)

Béla Győrffy, Member of the Hungarian Academy of Sciences (HAS), retired director and research professor of the Agricultural Research Institute of HAS, holder of the Széchenyi Award, Honorary Chairman of the Crop Production Committee of HAS, President of the Crop Production Society of the Hungarian Agricultural Association, Honorary President of the Soil Cultivation Society, Chairman of the Hungarian National Committee of the European Society of Agronomy, member of the International Biometric Society, the European Weed Science Society, the International Soil Science Society, the Scientific Qualification Committee and the National Committee for Technological Development, and Honorary Doctor of Szent István University, Debrecen University of Agricultural Sciences and the Georgikon Faculty of Agricultural Sciences of Veszprém University, died suddenly on May 8th 2002 in his 75th year. He taught many generations of scientists, was untiring in the organisation of international scientific activities and elaborated numerous new production technologies. His openness and his calm, unruffled wisdom will be missed by all who knew him. With his death, Hungarian agricultural scientific circles have lost one of their most outstanding members.

Béla Győrffy was born in Kemenesmagasi, a small village in Vas County, West Hungary, in 1928. Two other great figures of Hungarian agricultural science also came from this village: Andor Jánossy and László Berzsenyi-Janosits. He attended primary school in Kemenesmagasi and in Burgenland, and went to several secondary schools, first in Zalaegerszeg, then in Székesfehérvár, finally passing his school-leaving examination with distinction in the Agricultural School in Szarvas. He started his university studies at the József Nádor Technical and Economic University in Budapest and finished his degree at the University of Agricultural Sciences, which was its legal successor. He took with him to the university the high standard of farming he had experienced at home, together with an exceptionally wide-ranging cultural background. During his university years he was a member of the famous Győrffy College where he not only deepened his professional knowledge, but also broadened his outlook on literature, history, economics and ideology, learning from some of the best experts on these subjects, who often held quite opposing views. All this combined to make tolerance one of his most characteristic traits.

His first post was in the Department of Agricultural Policies at the University of Economics in 1948–1949, where he worked with Imre Nagy, who later became Prime Minister. In 1949 he went to the Timiryazev Academy in Moscow as a postgraduate student, where his supervisor was Harchenko, one of the most outstanding professors of the Pryanishnikov school. Most of the experiments required for his postgraduate work were carried out in Hungary, in Martonvásár, Óvár and Karcag. He defended his Ph.D. thesis, entitled "Double cropping in Hungary" in 1953. Béla Győrffy was greatly influenced by the results achieved in this field by Prof. János Surányi and especially by a saying of Surányi's: "Experience can replace many things, but nothing can replace experience". He obtained his D.Sc. in agricultural sciences in 1986.

He worked in the Agricultural Research Institute in Martonvásár from 1952 onwards, filling a number of posts, being deputy director from 1953 to 1956, then research associate, senior research associate, head of department, director from 1981 to 1988 and research professor from 1989 until his death. He had been chairman of the institute's Scientific Council ever since it was established. He had also carried out the duties of chairman of the Editorial Committee of the institute's periodical, "Martonvásár" from the first issue.

He was elected corresponding member of the Hungarian Academy of Sciences in 1987 and full member in 1992. He was chairman of the Academy's Supervisory Committee between 1990 and 1995 and was President of the Academy's Life Sciences Advisory Board from 1996 to 2001.

Béla Győrffy's working life covered a half century crowded with events that tested the physical and moral strength of those caught up in them. This young man from Kemenesmagasi belonged to the generation of young professionals who set to work to put Hungary's agriculture back on its feet after the second world war. The new outlooks created by his work made him one of the most prominent personalities in Hungarian agriculture.

The half-century of his active life is inseparable from crop production in Martonvásár. Together with his colleagues he elaborated some of the first technologies for up-to-date maize production systems. Between 1959 and 1961 he set up the first long-term crop production experiments in Hungary, which are today of inestimable value in research and education, serving as live field laboratories. These experiments were designed for the investigation of correlations between crop rotations, continuous cropping, fertilisation and soil tillage systems. He placed great emphasis on the interactions between genotype and environment and between genotype and technology. Much of his time was also spent examining the techniques widely applied in practice.

In a paper published in 1958 with the title "Revolution in maize production" he reported on results achieved with non-hoed maize production, which was the subject of much debate at the time. As time passed it became clear that his views on the subject were correct. In this connection he began to study the question of weed control and was one of the initiators of herbological research in Hungary. He was co-author of a number of herbicide patents. He had no doubt that the poet Dániel Berzsenyi was right when he wrote, "Untilled soil only grows weeds".

When it became clear that although Hungarian maize hybrids were competitive with American hybrids with respect to yield potential their stalk strength was unsatisfactory, he began to seek foreign hybrids for introduction into Hungary. Few people know that there was a time when more than half the maize-growing area of Hungary was sown to hybrids which Béla Győrffy had introduced.

He also encouraged his colleagues to strike a happy medium in the debate over chemisation and organic farming. "I am well aware," he wrote, "that although there is much to be said in favour of chemisation, many data indicate that there is another side to the coin. However, the solution is not to exile chemicals from agriculture, since this would lead to famine and unforeseeable consequences. The fact must be accepted that in modern civilisation neither medicines nor pesticides can be dispensed with. I am in agreement with those who, quite rightly, express the need for accommodating, environment-friendly agriculture or for sustainable agriculture."

The results of his research were published in over 150 scientific papers in Hungarian and other languages. The first three decades of his career coincided with a period in Hungarian agriculture when the country's crop production received recognition not only from the neighbouring countries, but also from developed Western countries. His active life made a substantial contribution to the development of crop production, tillage, nutrient management and chemisation in Hungary. His work had a decisive influence in laying down the theoretical basis of large-scale maize production, in introducing it into practice and in achieving its acceptance in professional circles.

Béla Győrffy was the director of the Agricultural Research Institute of the Hungarian Academy of Sciences for eight years, from 1981 to 1988. His management of scientific activities further enhanced the reputation of the institute both at home and abroad, not only in the field of breeding and crop production, but also as regards research on experimental biology.

In recognition of his work he was granted many awards. In 1962 he received the Academy Prize and in 1979 the Gold Labour Medal and the Cserháti Prize. He was twice awarded the gold grade of the Outstanding Inventor Prize and once the silver grade. In 1995 he received the Baross Memorial Medal and in 1997 the Széchenyi Prize.

In a film made in 1984, entitled "Where I was born – Where I work. A visit to Dr Béla Győrffy in Kemenesmagasi and Martonvásár", he expressed his research principles in the form of three quotations. The first came from the Bible, from the first epistle of St. Paul to the Thessalonians: "Prove all things; hold fast that which is good", the second was taken from Péter Veres: "Write about the present, which takes its origin from the past and progresses towards the future", while the third was an opinion expressed by János Kádár at a Party Conference in Budapest: "Scientists should besiege the heavens with their thoughts, but should keep their feet firmly on the ground".

Béla Győrffy had an exceptional personality, characterised by a constant search for a happy compromise which would serve to resolve conflicts and reconcile extremes. He was prepared to listen to everyone and weigh up their opinions before making decisions which were always moderate. He constantly encouraged us all to respect each other and to listen to what the other had to say. He was able to converse equally well with people in all walks of life, but perhaps he found the greatest pleasure in talking to young people. He was always pleased to see new young colleagues in Martonvásár, as he knew that the future of agricultural research was in their hands.

This much-travelled scientist from Vas County became a permanent member of the Martonvásár community with the natural simplicity of which only great people are capable. This is clearly illustrated by the fact that the local people made him an honorary citizen of Martonvásár in 1995. Those of us who lived and worked with him in Martonvásár know that he found his greatest pleasure in pruning his vines and tending his sheep. Such simple pleasures are only given to those who are able to maintain their integrity throughout a long life, like this son of a farmer from Kemenesmagasi.

O. VEISZ

Acta Agronomica Hungarica, 51(1), pp. 137–138 (2003)

Obituary

PROF. JÁNOS DOHY
(1934–2002)

The outstanding and widely-known animal scientist, János Dohy, died on 11 November 2002. His great knowledge and his willingness to give help and advice will be greatly missed by animal scientists, practical breeders and students in Hungary. His outstanding personality and integrity served as an example to many of us.

Prof. Dohy was born into a family where his grandfather and father were also well-known agricultural scientists. From them he inherited his devotion to nature and his love of the soil and farm animals, and of the people working with them.

After graduating from the University of Agricultural Sciences, Gödöllő he was invited to work as assistant to Prof. Arthur Horn. Between 1957 and 1964 Dr. Dohy worked intensively in the large-scale cattle crossing experiment, aimed at establishing a new Hungarian dairy cattle breed composed of 25% Danish Jersey and 75% Fleckvieh. Besides his work in this very successful breeding programme, he was engaged in research on the optimisation of heterosis, the definition of the type of dairy cow best suited to local conditions, and the investigation of factors determining the efficiency of production per unit area. For most of this period János Dohy worked in the Animal Breeding Research Centre in the team headed by Arthur Horn.

Between 1964 and 1974 Dr. Dohy was assistant professor at the University of Veterinary Sciences, Budapest. In 1974 he was appointed to the post of deputy general director at the Research Institute of Animal Breeding in Herceghalom.

In 1976 he moved to Kaposvár Agricultural College, where he was Director of the College, with responsibility for research activities.

In 1980, János Dohy became Head of the Animal Science Department at the University of Veterinary Sciences in Budapest, while in 1984 he accepted a similar post at the University of Agricultural Sciences in Gödöllő. Throughout his academic career he was active in both research, research organisation and international relations. He was a leading scientist in cattle breeding, applied genetics and biotechnology.

His teaching and scientific achievements were recognised by the award of many prizes and honorary medals in Hungary. He became a corresponding member of the Hungarian Academy of Sciences in 1993 and a full member in 1998. He was elected as vice president of the Agricultural Sciences Section of the Hungarian Academy of Sciences in 1996, and became president of this section from 1999 till 2002. He received the two most prestigious prizes for scientific merit in Hungary, the Academy Prize (1976) and the Széchenyi Prize

(1996), as well as several others. He was Doctor Honoris Causa of Pannon Agricultural University (1994) the Agricultural University of Debrecen (1997), the University of West Hungary (2001), and the University of Kaposvár (2002).

He was very active in international scientific circles, and he had an excellent knowledge of English, German and Russian. He visited more than 30 countries, giving lectures, attending congresses and working as a consultant.

He was a regular speaker at EAAP meetings, giving a total of 38 presentations, and also helped to organise EAAP meetings in Hungary (1970, 1986, 2001). He was member of the EAAP council from 1982–1988, and was proud to be presented with the "Distinguished Service Award" in 1999. He was "Ehrenmitglied der Deutschen Gesellschaft für Züchtungskunde" (1997) and became corresponding member of the "Academia dei Georgofili, Firenze" in 2001.

János Dohy was the author or co-author of more than 550 papers, over 200 of which were published in scientific journals in Hungarian, German, English and Russian. He was dedicated to transmitting research results to people working in practice. He was the author, co-author and editor of 16 books and chapters published in Hungary and abroad. He was a member of the editorial board of the scientific journals Acta Agronomica Hungarica, Hungarian Agricultural Research, Hungarian Journal of Animal Breeding and Nutrition, Egyptian Journal of Animal Science, Animal Science Papers and Reports of the Polish Academy of Science, and Livestock Production Science.

Professor János Dohy left us as unassumingly as he lived. We have lost a great personality, a true friend who will be sorely missed.

P. HORN

MAGYAR
TUDOMÁNYOS AKADÉMIA
KÖNYVTÁRA

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ACTA AGRONOMICA HUNGARICA publishes papers, short communications, review articles and book reviews of international interest in the field of **basic and applied research in agronomy,** chiefly on the physiology, genetics, breeding and production of cultivated crops. Only original papers will be published. A copy of the Publishing Agreement will be sent to the authors of papers accepted for publication; manuscripts will be processed only after receiving a signed copy of the agreement.

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Manuscripts and editorial correspondence should be addressed to

Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
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Phone: (36-22) 569-521
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GENETIC ANALYSIS OF YIELD AND ITS COMPONENT TRAITS IN SPRING WHEAT

S. K. JOSHI, S. N. SHARMA, D. L. SINGHANIA and R. S. SAIN

ALL INDIA COORDINATED WHEAT AND BARLEY IMPROVEMENT PROJECT, RAJASTHAN AGRICULTURE UNIVERSITY, AGRICULTURAL RESEARCH STATION, DURGAPURA, JAIPUR, RAJASTHAN, INDIA

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Combining ability analysis in spring wheat (*Triticum aestivum* L. em. Thell) involving 10 diverse parents and their 45 F_1 and F_2 progenies indicated significant differences between the parents for GCA and between the crosses for SCA for all the characters studied. The GCA and SCA components of variance were significant for all the traits. However, the GCA component of variance was predominant, indicating the predominance of additive gene effects for the traits studied. Among the parents HD 2329, Raj 1972, HD 2285 and HD 2428 were the best general combiners for grain yield and average to high combiners for other important traits. The best specific crosses for grain yield were CPAN 3004 × Durgapura 65, Sonalika × HD 2329, Raj 3077 × CPAN 3004, Raj 3077 × HD 2428 and HD 2428 × WH 157. The parent Raj 1972 was the best general combiner for grain yield and protein content, while Raj 3077 and Lok-1 were the best general combiners for protein content. The most suitable specific crosses for protein content were HD 2329 x HD 2285, HD 2428 × Raj 1972 and CPAN 3004 × WH 157. Most of the specific crosses for grain yield and protein content involved high × average, average × average or average × poor general combiners. To ensure a further increase in grain yield along with high protein, combinations of desirable yield components are advocated. The exploitation of additive and non-additive gene actions through bi-parental mating and/or diallel selective mating systems are suggested for a tangible advance in grain yield coupled with high protein in spring wheat.

Key words: bread wheat, variety, gene effects, heterosis, yield components

Introduction

Wheat (*Triticum* spp.), the second most important cereal crop in India, occupies 27 million hectares with a total production of about 70 million tonnes, in comparison to 12.3 million tonnes in 1965. Wheat production in India has been hovering around the 70 million tonnes mark during the last five years, placing it as the largest wheat producer in the world after China, with a growth rate of approximately 3% per annum (Nagarajan, 2001). The continual introduction of new improved varieties has pushed wheat productivity to new levels. The increase in productivity, however, has not been uniform all over the country, and this indicates the opportunities and areas for increasing wheat production in the future. To keep pace with the burgeoning population, growing at 1.8% per annum, and with the changing food habits of the rural and urban populace, it is necessary to further increase the productivity level. To sustain this production level it is necessary to overcome biotic and abiotic stresses through the development of new sets of varieties. The grain quality of wheat for the domestic and international market must also be improved to fetch higher prices on the market. This calls for alternative selection strategies in wheat breeding.

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In recent years, there has been an increase in the cropping intensity due to the availability of irrigation, power and fertilizer inputs in India. The farmers are now successfully cultivating two or three crops a year. Such systems of multiple cropping need varieties of different durations. Rice-wheat, which is one of the major cropping systems, occupying about 10.5 million hectares, needs high-yielding short duration varieties. Moreover, farmers in the North Western and North Eastern Plain Zones have also started growing wheat after cotton, mustard, sugarcane (ratoon) and potato, in the month of December even up to January. To achieve 100% cropping intensity, spring wheat as double crop is preferred under late-sown conditions. However, there are yield losses due to high temperature in warmer areas (Howard, 1924; Fischer and Maurer, 1976; Shpiler and Blum, 1986; Hu and Rajaram, 1994; Wardlaw, 1994; Stone and Nicolas, 1995), so the development of early maturing heat-tolerant wheat varieties coupled with high yield has become one of the most important aspects of breeding nowadays. The experimental evidence clearly indicates that normal-sown varieties are generally not suitable for the late-sown situation and vice versa and a separate breeding programme is needed for each situation (Rajaram, 1988). Thus, various plant breeding tools are becoming increasingly important in the changed scenario.

It is becoming increasingly clear that a yield plateau has been reached and that only a sound, systematic breeding programme will bring a breakthrough in grain yield. Attempts must be made to explore the possibility of novel genes for the inheritance of desirable traits. Improving the genetic yield potential of varieties of crop plants depends on a largely improved breeding population. To improve the breeding population a thorough knowledge of gene action, the heritability of the characters and the genic contents of the parents is needed. This emphasizes the necessity of testing the parents for combining ability. The isolation of superior and transgressive segregates equal or superior to the F_1 in advanced generations depends upon the type of gene action predominantly responsible for the expression of the characters. Several mating designs and models, which provide estimates of the effects and variances due to combining ability, are available and each of them has its advantages and disadvantages. The diallel method of analysis was developed in order to provide information on the genetic architecture of the breeding material, which helps in the identification of the parents / crosses to be included in a future breeding programme for tangible crop advancement. Diallel analysis also provides a unique opportunity to test a number of lines in all possible combinations. Thus, the main objective of the present studies was to identify the best combining parents and their crosses on the basis of their general and specific combining ability for yield and its component traits in a normally sown environment for the further amelioration of grain yield in bread wheat.

Materials and methods

Ten varieties of spring wheat (*Triticum aestivum* L. em. Thell), namely, Raj 3077, CPAN 3004, HD 2428, Lok-1, Durgapura 65, Raj 1972, Sonalika, HD 2329, HD 2285 and WH 157, were selected for the present study. Varieties Raj 3077, HD 2285 and Sonalika were included because they were released for late and very late sowing situations in India and occupy a large area under late sowing conditions in various cropping systems, particularly in the rice-wheat system. These varieties have excellent genetic ability to thrive well in a heat stress environment through the manipulation of various yield components to produce good yield levels within a 90–100-day maturity period. All the ten parents were crossed in all possible combinations excluding reciprocals. The 10 parents and their resulting 45 F_1s and 45 F_2s were grown in a randomized block design with three replications, planted at the normal sowing date (20th November) at the Agricultural Research Sub-Station, Tabiji, Ajmer, Rajasthan, India. Plots of parents and F_1s consisted of four rows 3 m in length, while each plot of F_2 consisted of eight rows with a spacing of 30 cm between rows and 15 cm between plants. Twenty competitive plants were selected randomly from the parents and F_1s and 60 plants from the F_2 progenies to record observations on twelve characters, namely: days to 75% heading, days to 75% maturity, plant height (cm), flag leaf area (cm^2), tillers per plant, spike length (cm), grain yield per spike (g), grains per spike, 1000-grain weight (g), harvest index (%), grain yield per plant (g) and protein content (%) separately. Six random samples of seeds from each parent and from every cross in both the generations were ground and used for protein estimation. The protein content of the grain was estimated by the micro-Kjeldahl method. The percentage of protein was obtained by multiplying the percentage of nitrogen by a factor of 6.25.

The mean of each plot was used for statistical analysis. The data were first subjected to the usual analysis for a randomized block design (Panse and Sukhatme, 1967). The combining ability analysis was done following Method 2, Model 1 of Griffing (1956). Heterosis was estimated as the deviation of the F_1 value from the mid-parent and the better parent values.

Results and discussion

Analysis of variance indicated significant differences between the parents for all the characters. Similarly, the differences between the F_1 hybrids and F_2 progenies were found to be significant for all the traits, indicating the presence of diversity in the material (Table 1). Significant differences between parent vs F_1s and parent vs F_2s were also found for all the traits except for days to maturity and spike length in the parent vs F_1s, revealing the existence of heterosis and inbreeding depression. Significant differences between the genotypes for grain yield and related traits in different sets of material were also reported by Singh and Paroda (1985), Singh (1988) and Menon and Sharma (1997).

Analysis of variance for combining ability revealed that the variance due to general combining ability (GCA) and specific combining ability (SCA) was highly significant for all the traits studied in both the F_1 and F_2 generations. Thus, both kinds of gene effects were important in controlling the inheritance of all the characters studied. However, the GCA : SCA ratio normally tilted in favour of GCA for all the traits except 1000-grain weight in both the F_1 and F_2 generations, indicating the preponderance of additive gene effects in the genetic control of the traits. The 1000-grain weight was primarily controlled by non-additive components of genetic variance (Table 2). The present findings thus

Table 1
Analysis of variance showing mean squares for parents, F_1s and F_2s for different characters in a normal sowing environment

Character	Source									
	Rep	Parents	F_1s	P vs F_1s	Error	Rep	Parents	F_2s	P vs F_2s	Error
d.f.	2	9	44	1	100	2	9	44	1	100
Days to heading	0.71	169.48**	125.44**	4.82**	0.79	0.98	169.48**	69.05**	6.53**	0.93
Days to maturity	0.64	217.64**	199.73**	36.59	0.89	1.70	217.65**	186.55**	2294.15**	0.62
Plant height	0.51	214.95**	247.19**	866.30**	31.25	0.44	214.96**	321.90**	929.89**	28.30
Flag leaf area	0.25	26.62**	28.10**	5.98**	0.39	0.21	26.63**	12.02**	44.09**	0.38
Tillers per plant	0.04	2.40**	3.98**	3.88**	0.28	0.10	2.40**	3.05**	6.39**	0.35
Spike length	0.12	7.23**	3.54**	0.27	0.23	0.60	7.23**	4.76**	6.60**	0.46
Grain yield per spike	0.03	0.220**	0.46**	2.35**	0.02	0.01	0.20**	0.52**	1.21**	0.02
Grains per spike	4.01	164.97**	117.64**	48.60**	4.41**	0.89	164.97**	108.03**	127.95**	6.33
1000-grain weight	0.74*	141.20**	104.65**	186.68**	0.11	1.09*	141.20**	110.24**	135.52**	0.18
Harvest index	0.05	156.90**	96.83**	706.87**	0.62	0.09	156.90**	68.82**	572.14**	0.42
Grain yield per plant	0.77	8.65	8.33	35.79	0.13	0.28	8.65**	9.23**	27.80**	0.15
Protein content	0.65*	3.79**	3.12**	3.94**	0.03	0.62**	3.79**	3.93**	4.39**	0.03

*, ** Significant at the 5% and 1% levels, respectively

Table 2
Analysis of variance showing mean squares for combining ability in a normal sowing environment

Characters	Source							
	GCA		SCA		Error		GCA : SCA	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
d.f.	(9)		(45)		(108)			
Days to heading	178.9**	123.4**	16.4**	17.9**	0.26	0.32	10.8:1	6.8:1
Days to maturity	247.9**	188.9**	30.2**	54.5**	0.30	0.21	8.1:1	3.4:1
Plant height	199.7**	124.2**	61.3**	101.2**	10.41	9.44	3.2:1	1.2:1
Flag leaf area	11.6**	11.1**	8.6**	3.8**	0.13	0.12	1.3:1	2.9:1
Tillers per plant	1.5**	0.8**	1.0**	0.6**	0.10	0.08	1.5:1	1.2:1
Spike length	2.6**	3.7**	1.1**	1.3**	0.10	0.15	2.3:1	2.8:1
Grain yield per spike	0.1**	0.2**	0.2**	0.1**	0.00	0.00	1.0:1	1.5:1
Grains per spike	50.6**	45.1**	39.5**	38.1**	1.47	2.11	1.2:1	1.1:1
1000-grain weight	33.2**	34.1**	38.2**	39.5**	0.04	0.06	0.8:1	0.8:1
Harvest index	104.9**	67.5**	26.2**	23.6**	0.21	0.14	3.9:1	2.5:1
Grain yield per plant	4.3**	4.8**	2.6**	2.8**	0.04	0.04	1.5:1	1.6:1
Protein content	3.2**	2.8**	0.6**	1.0**	0.01	0.00	5.1:1	2.8:1

** Significant at the 1% level

supported the reports of Singh and Rana (1987), Raghuvanshi et al. (1988), Singh (1988) and Pokhrel et al. (1993), clearly indicating that additive genetic variance was the main component of genetic variance in various economic traits in bread wheat. However, the preponderance of non-additive effects (Sharma and Singh, 1983; Singh, 1989; Menon and Sharma, 1997) and the role of both additive and non-additive effects were also reported for grain yield and its component characters (Dasgupta and Mondal, 1988; Menon and Sharma, 1995; Bhavasar et al., 1996; Sheikh et al., 2000; Singh, 2002) in wheat.

The estimates of general combining ability (GCA) effects revealed that among the parents HD 2329, Raj 1972, HD 2285 and HD 2428 were the best general combiners for grain yield and good to average combiners for most of the yield component characters (Table 3). However, the rest of the parents were poor combiners for grain yield and average to poor general combiners for most of the yield contributing traits. Parents Lok-1 and Sonalika were the best general combiners for early heading and maturity. Very similar trends were observed for the general combining ability of the parents in both the F_1 and F_2 generations. Parent HD 2329 was also a good general combiner for early heading and maturity, dwarfness, flag leaf area, spike length, grain yield per spike, 1000-grain weight and harvest index. Raj 1972 was a good combiner for early maturity, dwarfness, spike length, grain yield per spike, grains per spike, 1000-grain weight and protein content. HD 2285 was the best general combiner for early maturity, dwarfness, flag leaf area, grain yield per spike, 1000-grain weight and harvest index. Parent HD 2428 was also a good combiner for early heading and maturity, spike length, grain yield per spike, grains per spike and harvest index.

Table 3
Estimates of general combining ability effects for different characters in a normal sowing environment

Parent	Days to heading		Days to maturity		Plant height		Flag leaf area		Tillers per plant		Spike length	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Raj 3077	–0.70**	0.93**	–1.75**	0.26**	0.69	–0.25	0.70**	0.12**	0.29**	–0.07**	0.18**	0.04**
CPAN 3004	3.09**	2.70**	1.79**	2.23**	1.20	2.52**	–1.21**	–1.23**	–0.12	0.08**	–0.27**	–0.06**
HD 2428	–2.81**	–1.08**	–4.00**	–1.30**	0.20	–2.44**	–0.46**	0.24**	–0.46**	–0.11	0.64**	0.58**
Lok- 1	–1.01**	–0.83**	–2.13**	–0.81**	5.75**	3.54**	–0.94**	0.14**	0.15*	0.01	0.38**	–0.56**
Durgapura 65	1.11**	0.88**	0.94**	–0.26**	7.12**	4.32**	0.54**	1.17**	0.30**	0.21*	–0.68**	–1.11**
Raj 1972	0.20	–0.56**	–0.50*	–1.05**	–4.94**	–1.52*	–0.29**	–1.35**	–0.02	0.06**	0.27**	0.27**
Sonalika	–3.58**	–2.31**	–3.50**	–4.10**	0.32	1.41*	–1.21**	–1.06**	–0.82**	–0.54**	–0.08**	0.06**
HD 2329	–4.84**	–5.51**	–3.35**	–4.46**	–4.09**	–4.45**	1.15**	0.62**	–0.10	0.01	0.10**	0.33**
HD 2285	–0.27	–0.92**	0.93**	–0.33**	–1.82*	–4.98**	1.59**	1.37**	0.20**	–0.06**	–0.49**	–0.33**
WH 157	8.81**	6.70**	11.57**	9.82**	–4.42**	1.63**	0.13**	0.47**	0.53**	0.41**	0.71**	0.80**
SE (gi) ±	0.019	0.020	0.020	0.015	0.780	0.700	0.099	0.096	0.070	0.086	0.081	0.011
SE (gi-gj)	0.044	0.050	0.050	0.035	0.170	0.150	0.022	0.021	0.015	0.019	0.080	0.026

	Grain yield per spike		Grains per spike		1000-grain weight		Grain yield per plant		Harvest index		Protein content	
Raj 3077	0.05**	0.07**	0.75**	0.54**	0.85**	–0.13**	–0.20**	–0.25**	2.23**	1.81**	0.38**	0.07**
CPAN 3004	0.08**	–0.02**	–1.35**	0.30*	0.19**	–0.88	–0.33**	–0.34**	–0.39**	–0.41**	–0.28**	0.11**
HD 2428	0.04**	0.02**	0.48**	2.32**	0.42*	–1.41**	0.11**	0.05**	1.36**	1.44**	–0.44**	0.16**
Lok-1	–0.20**	–0.27**	–3.65**	–2.85**	–1.04**	–1.91**	–0.08**	–0.42**	0.45**	–0.92**	0.49**	0.49**
Durgapura 65	–0.21**	–0.09**	–1.11**	–2.99**	2.24**	0.08**	–0.09**	0.86**	–1.88**	–1.01**	0.01**	–0.04**
Raj 1972	0.15**	0.19**	0.92**	0.77**	1.04**	2.84**	0.98**	1.05**	–2.78**	–1.96**	0.45**	0.16**
Sonalika	0.00	0.09**	1.62**	–0.84**	–0.01**	–0.31**	–1.16**	–1.08**	–5.45**	–4.57**	–1.11**	–1.25**
HD 2329	0.04**	0.18**	–2.14**	–0.88**	2.79**	2.78**	0.85**	0.84**	2.59**	1.41**	–0.18**	–0.14**
HD 2285	0.05**	0.04**	1.09**	0.74**	0.87**	0.51**	0.06**	0.48**	4.80**	4.07**	0.61**	0.47**
WH 157	0.01**	–0.02**	3.40**	2.98**	–2.87**	–2.30**	–0.14**	–0.31**	–0.94**	0.14**	–0.02**	–0.03**
SE (gi) ±	0.000	0.000	0.110	0.150	0.030	0.004	0.032	0.003	0.000	0.010	0.050	0.000
SE (gi-gj)	0.000	0.000	0.240	0.350	0.067	0.001	0.071	0.008	0.034	0.020	0.000	0.000

*, ** significant at the 5% and 1% levels, respectively

Apparently, therefore, there is still further scope for improving the combining ability for component traits, as none of the high combiners for grain yield was a high combiner or at least an average combiner for all the desirable traits. Among the other parents Raj 3077 was a high combiner only for flag leaf area, spike length, grains per spike and protein content, Durgapura 65 for flag leaf area, tillers per plant and 1000-grain weight, Sonalika for early heading and maturity, WH 157 for flag leaf area, tillers per plant, spike length and grains per spike, and Lok-1 for early heading, early maturity and protein content (Table 3). Parent CPAN 3004 did not show consistency as a good combiner in both the generations, but was observed to be a good combiner for grain yield per spike and 1000-grain weight in the F_1 and for tillers per plant, grains per spike and protein content in the F_2 generation.

High GCA effects are mostly due to additive gene effects or additive × additive interaction effects, as earlier reported by Griffing (1956). In view of this, breeders may utilize the good general combiners in specific breeding programmes for improving the grain yield of wheat. It thus seems feasible that the GCA rank for grain yield is related to the GCA for useful yield components. Breeders are therefore recommended to breed for superior combining ability for the component traits with the ultimate objective of improving the overall GCA for grain yield in spring wheat. The parents HD 2329, Raj 1972, HD 2285 and HD 2428 could be utilized extensively in the hybridization programme to accelerate the pace of genetic improvement of grain yield in spring wheat in a heat stress environment.

In order to synthesize a dynamic population accumulating most of the favourable genes, use could be made of the aforesaid parents, which are good general combiners for several characters, in multiple crossing programmes. Apart from conventional breeding methods, based on the additive or additive × additive type of gene action, population improvement appears to be a hopeful alternative. The diallel selective mating system (Jensen, 1970) is a useful technique, which delays the rapid fixation of gene complexes, permits the breakdown of linkage, and generally promotes the recombination and concentration of favourable genes or gene complexes into the central gene pool, by a series of multiple crosses.

Normally the SCA effects do not contribute tangibly to improvement in self-fertilizing crops, except where the commercial exploitation of heterosis is feasible. The SCA effects represent dominance and epistatic interaction, which may be related to heterosis. However, in self-pollinated crops like wheat, the additive × additive type of interaction component is fixable in later generations. Breeders thus aim to obtain transgressive segregants through crosses and to produce more potent homozygous lines. Jinks and Jones (1958) emphasized that the superiority of the hybrids might not be indicated by their ability to yield transgressive segregants, and that SCA would provide a more satisfactory criterion.

The estimates of specific combining ability (SCA) revealed that out of 45 crosses, 26 were good specific combiners for grain yield in F_1 and 24 in the F_2. It is noteworthy that 21 crosses showed positive and significant SCA effects for grain yield in both the F_1 and F_2 generations. Generation effects were also noticed in the SCA of the crosses. The highest positive significant SCA effect was exhibited by the cross CPAN 3004 × Durgapura 65 in both generations. Other good combinations, which showed significant SCA effects for grain yield in both generations, were Sonalika × HD 2329, Raj 3077 × CPAN 3004, Raj 3077 × HD 2428, HD 2428 × WH 157, CPAN 3004 × HD 2329, Raj 3077 × Raj 1972, HD 2428 × Durgapura 65, Durgapura 65 × Sonalika and Lok-1 × Durgapura 65. These crosses had higher yields and in most of the crosses one of the parents involved was a good combiner, indicating that such combinations could be expected to give rise to desirable transgressive segregants. All the best crosses for grain yield also showed average to high SCA effects for most of the yield components. It is therefore recommended that these crosses should be used in future breeding programmes for recombining the desirable traits in the envisaged elite genotypes.

The gliadin protein (a constituent of gluten) probably accounts for much of the baking differences observed between cultivars. In India wheat is used for human consumption mainly in the form of chapatti, so cultivars with high baking quality are needed. To improve the protein content of wheat, the parents Raj 1972, Raj 3077 and Lok-1 could therefore be used in breeding programmes. The crosses Durgapura 65 × HD 2329, CPAN 3004 × WH 157, HD 2428 × HD 2329, Raj 1972 × WH 157, HD 2428 × Raj 1972 and HD 2329 × HD 2285 had high positive SCA effects. For a tangible advance in protein content, due attention should be given to these crosses in order to develop new varieties having higher protein levels for specific industrial uses.

It is noteworthy that crosses which exhibited consistently positive SCA in both generations also exhibited positive significant heterosis. Thus, the results of the present study indicated some relationship between SCA effects and heterosis. It is therefore suggested that SCA performance could be considered as a criterion for selecting the best crosses in bread wheat. It may also be worthwhile to attempt bi-parental mating in the segregating generation between selected crosses to permit superior recombinations. All the important crosses involving parents with high × average, average × average and average × poor general combiners indicated that the non-additive types of gene action, which are unfixable in nature, were involved in the selected cross combinations.

The study demonstrates that both the additive (fixable) and non-additive (non-fixable) components of genetic variance were involved in governing the inheritance of almost all the quantitative and qualitative traits, although additive genetic variance was predominant. Therefore, bi-parental mating and/or diallel selective mating, which may allow the intermating of selected genotypes in different cycles and the exploitation of both additive and non-additive gene effects, could be useful for the genetic improvement of the characters of spring wheat. The inclusion of F_1 hybrids showing high SCA and having parents with good GCA, in multiple crosses, could also prove a worthwhile approach for tangible improvements in the grain yield of spring wheat.

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PHOTOSYNTHETIC ATTRIBUTES AND SEED YIELD OF SUNFLOWER (*Helianthus annuus* L.) AS INFLUENCED BY DIFFERENT LEVELS AND RATIOS OF NITROGEN AND PHOSPHORUS FERTILIZERS

N. THAVAPRAKASH, G. SENTHILKUMAR, S. D. SIVAKUMAR and M. RAJU

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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A field experiment was conducted at the Main Research Station, University of Agricultural Sciences, Dharwad, India, on medium black soils during the *kharif* (wet) season of 1999. The experiment was laid out in a randomized complete block design with varying N/P ratios (0.67 to 2.00) along with a control with a constant level of potassium (60 kg ha^{-1}). The results revealed that the number of green leaves plant^{-1}, the dry matter accumulation in the leaves, leaf area (dm^2 plant^{-1}) and leaf area index (LAI) increased up to the flowering stage (65 DAS) and thereafter declined. In the early stages (seedling and button stages) there was no significant variation with respect to the number of green leaves plant^{-1} among the treatments except in the control. Similarly, leaf area and LAI did not vary at the seedling stage. Treatments receiving N/P ratios of >1.0 or 1.0 with higher doses of nitrogen (120 kg N ha^{-1}) gave a significantly higher number of green leaves plant^{-1}, leaf area and LAI as compared to N/P ratios of <1.0 and the control in later stages. The dry matter accumulation in the leaves (g plant^{-1}) differed in all the stages, but higher values were recorded in these same treatments. Thus, due to the higher number of green leaves, higher LAI and greater dry matter accumulation in the leaves, the treatments with an N/P ratio of >1.0 or 1.0 with 120 kg N ha^{-1} produced higher seed yields (3188 to 3554 kg ha^{-1}) than other N/P ratios (2761 to 3009 kg ha^{-1}). The highest yield (3554 kg ha^{-1}) was recorded with an N/P ratio of 1.0 in the treatment receiving 120 kg N and 120 kg P_2O_5 ha^{-1}. The correlation coefficients between these photosynthetic attributes and seed yield were also positive and significant.

Key words: N/P ratio, photosynthetic attributes, seed yield, sunflower

Introduction

Sunflower is an important oil seed crop in India. The crop was introduced to India in the early seventies and is gaining popularity with farmers due to its desirable attributes such as short duration, wide adaptability to soil and climatic conditions, photo- and thermo-insensitiveness, drought tolerance and higher oil yield per unit area than other oil seed crops. Though the crop has become familiar to the farming community, the yield levels are low (549 kg ha^{-1}) compared to the world average (1233 kg ha^{-1}). The low productivity of sunflower in India is due to the fact that the crop is mainly concentrated on marginal lands under rainfed conditions with poor fertility management and inadequate plant protection measures. Optimum fertilizer management can result in a twofold increase in the seed yield of sunflower (Chorey and Thosar, 1997). Several studies on the nutrient requirements of sunflower indicated that, among the macro-nutrients, nitrogen and phosphorus are likely to be the primary limiting nutrients in sunflower under most environments (Devidayal and Agarwal, 1998; Dhoble, 1998; Mallikarjuna et al., 2000).

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Photosynthetic attributes, such as number of green leaves plant^{-1}, leaf area, LAI and dry matter accumulation, are considered as important parameters which must be maintained properly for better production in sunflower. Earlier studies (Megur, 1998; Hittinahalli, 1998) demonstrated that higher dry matter accumulation in the leaves and LAI produced higher yields in sunflower. However, there is little or no information available regarding the effect of nitrogen, phosphorus and their ratios on the photosynthetic attributes of sunflower. Hence, the present study was planned and carried out to assess the effect of nitrogen and phosphorus levels and ratios on the photosynthetic attributes and seed yield of a sunflower hybrid.

Materials and methods

The field experiment was conducted during the *kharif* (wet) season (June–September) of 1999 at the Main Research Station, University of Agricultural Sciences, Dharwad. The experimental site is situated at 15° 26' N latitude, 75° 07' E longitude and at an altitude of 678 m above mean sea level. The soil was medium black clay in texture with a pH of 7.7. The soil was medium in organic carbon (0.62%), low in available nitrogen (270.61 kg N ha^{-1}), medium in available phosphorus (40.85 kg P_2O_5 ha^{-1}) and high in available potassium (436.57 kg K_2O ha^{-1}), as estimated by the wet oxidation method (Jackson, 1967), the alkaline permanganate method (Subbiah and Asija, 1956), Olsen's method (Muhr et al., 1965), and the flame photometer method (Muhr et al., 1965), respectively. Sunflower hybrid DSH-1, with a field duration of 90 days, was used in the trial.

The experiment was laid out in a randomized complete block design (RCBD) with four replications on a gross plot size of 7.2 × 4.2 m and a net plot size of 6.0 × 3.6 m. The experiment involved nine treatments (Table 1) with varying N/P ratios (0.67 to 2.00) along with a control, achieved by keeping the potassium level constant at 60 kg K_2O ha^{-1}. Seeds pre-treated with fungicide (metalaxyl + mancozeb @ 4 g kg^{-1} seed) were hand dibbled with a spacing of 60 × 30 cm. The fertilizers nitrogen, phosphorus and potassium were applied in the form of urea, di-ammonium phosphate and muriate of potash, respectively. Fifty per cent of the nitrogen and the entire quantity of phosphorus and potassium required for each treatment were applied at the time of sowing. The top dressing (in band placement) of nitrogen in the form of urea was done at 40 days after sowing (DAS). The plots were kept weed free by integrated weed management practices involving pre-emergence application of metolachlor (30 EC) @ 1.0 kg ha^{-1} with two intercultivations (20 and 35 DAS) and one hand weeding at 30 DAS. Plant protection measures were carried out to control the pests. The crop was harvested when it attained maturity.

To record various biometric observations on sunflower, a sample consisting of five plants was selected at random from each net plot and the number of green leaves plant^{-1}, dry matter accumulation (g plant^{-1}), leaf area and LAI were measured at the seedling (25 DAS), button (45 DAS), flowering (65 DAS), seed formation (78 DAS) and maturity (87 DAS) stages. The number of green leaves plant^{-1} was recorded by counting the number of fully opened green leaves on the main stem of a plant. Dried and yellow-coloured leaves were not included in the count. The oven-dry weight (drying at 70°C to constant weight) of leaves at different growth stages was recorded to determine the dry leaf weight. Leaf area (dm^2 plant^{-1}) was measured as described by Vivekanandan et al. (1972). A non-destructive plant canopy analyser (LAI 2000, manufactured by LI-COR, Lincoln, Nebraska, USA) was used to measure LAI. The seed yield from each net plot was recorded and expressed in kg ha^{-1}. The data were subjected to statistical analysis (Gomez and Gomez, 1984). The treatment means were compared using Duncan's multiple range test (DMRT). The data were also used to calculate the correlation coefficient between photosynthetic attributes and the seed yield of sunflower. Their direct and indirect effects were observed.

Results and discussion

Seed yield

The seed yield of sunflower differed significantly due to N and P fertilizers (Table 1). Treatments receiving an N/P ratio of >1.0 or 1.0 produced higher seed yield (2875 to 3554 kg ha^{-1}) than other N/P ratios (2761 to 3009 kg ha^{-1}) and the control (1949 kg ha^{-1}). Further, among the better treatments, an N/P ratio of 1.0 or >1.0 with the 120 kg N ha^{-1} dose produced a better seed yield (3188 to 3554 kg ha^{-1}) than lower (75 kg ha^{-1}) doses of N (2875 to 3009 kg ha^{-1}). The best results were obtained in the treatment with an N/P ratio of 1.0 and a double dose of N and P. The higher yields of sunflower in these treatments might be due to the translocation of nutrients in the presence of more nitrogen. Higher seed yield at an N/P fertilizer ratio of >1.0 was documented by many workers (Sarmah et al., 1992; Singh and Singh, 1997; Devidayal and Agarwal, 1998; Baldev Raj et al., 1999).

Photosynthetic attributes

Higher seed yield was mainly due to the improvement of photosynthetic attributes in the above treatments. The photosynthetically active parts of sunflower, such as number of green leaves, dry matter accumulation in leaves, leaf area and LAI, differed significantly at various nitrogen and phosphorus ratios (N/P ratios).

Table 1

Seed yield (kg ha^{-1}) and number of green leaves plant^{-1} of sunflower at different growth stages as influenced by nitrogen and phosphorus fertilization

No.	Treatments (kg ha^{-1})				Seed yield	Number of green leaves plant^{-1}				
	N	P_2O_5	K_2O	N/P_2O_5		25 DAS	45 DAS	65 DAS	78 DAS	87 DAS
1	0	0	60	–	1949e*	5.8b	20.9b	24.3c	9.9c	3.7c
2	60	75	60	0.80	2800d	7.0a	24.8a	25.6b	11.9b	6.2b
3	60	90	60	0.67	2761d	7.1a	23.8a	25.9b	11.9b	6.6b
4	90	75	60	1.20	3009cd	7.0a	24.8a	26.6b	12.1b	6.7b
5	90	90	60	1.00	2875d	7.2a	24.9a	26.6b	12.1b	6.8b
6	120	60	60	2.00	3188bc	7.3a	24.2a	28.0a	15.0a	8.1a
7	120	75	60	1.60	3397ab	7.3a	24.0a	28.1a	15.0a	8.1a
8	120	90	60	1.30	3220bc	7.4a	24.9a	28.2a	14.8a	8.2a
9	120	120	60	1.00	3554a	7.5a	24.9a	28.3a	15.1a	8.3a
CD (P=0.05)					252	0.9	1.3	1.2	1.7	0.9

25 DAS seedling stage, 45 DAS: button stage, 65 DAS: flowering stage, 78 DAS.: seed formation stage, 87 DAS: maturity stage; DAS: days after sowing. *In a column mean values followed by the same letter do not differ significantly at P=0.05 by DMRT

Number of green leaves plant^{-1}

The number of green leaves plant^{-1} increased as crop growth advanced until flowering and thereafter decreased (Table 1). In the early stages (seedling and button stages) there were no significant differences between the treatments except for the control. In later stages, however, treatments receiving N/P ratios of >1.0 or 1.0 with higher doses of nitrogen (120 kg N ha^{-1}) gave a higher number of green leaves plant^{-1} than N/P ratios of < 1.0 or the control. This might be due to the higher doses of nitrogen, which allowed the plants to retain a greater number of leaves. These results are in accordance with the results of several workers (Sharma, 1994; Tomar et al., 1997; Narayana and Patel, 1997).

Dry matter accumulation in leaves

Dry matter accumulation in the leaves was significantly influenced by nitrogen and phosphorus fertilization at all the stages (Table 2). Higher dry matter accumulation in the leaves was obtained at the flowering stage than in the other growth stages. Irrespective of the stage, treatments involving an N/P ratio of >1.0 or 1.0 with 120 kg N ha^{-1} led to a greater accumulation of dry matter in the leaves as compared to N/P ratios of < 1.0 and the control. More specifically, at the flowering stage there was more dry matter accumulation in the leaves (54.83 to 55.54 g plant^{-1}) in the above treatments than in the other ratios (49.14 to 50.13 g plant^{-1}) or the control (35.11 g plant^{-1}). The accumulation of more dry matter in the leaves in treatments with N/P ratios of >1.0 or 1.0 might have provided more leaf area plant^{-1}, thus improving the supply of photosynthates and in turn increasing the seed yield. The increased accumulation of dry matter in the leaves due to the application of nitrogen and phosphorus fertilizers with an N/P ratio of 1.0 was also noted by Megur (1988) and Sarmah et al. (1992).

Table 2

Leaf dry matter (g plant^{-1}) of sunflower at different growth stages as influenced by nitrogen and phosphorus fertilization

No.	Treatments				Leaf dry matter				
	N	P_2O_5	K_2O	N/P_2O_5	25 DAS	45 DAS	65 DAS	78 DAS	87 DAS
	(kg ha^{-1})								
1	0	0	60	–	0.77c*	17.74c	35.11c	13.80c	6.20c
2	60	75	60	0.80	1.69b	25.11b	49.14b	22.48b	11.43b
3	60	90	60	0.67	1.69b	25.92b	49.97b	22.45b	11.78b
4	90	75	60	1.20	1.70b	26.49b	50.13b	22.59b	11.36b
5	90	90	60	1.00	1.71b	26.30b	49.95b	22.65b	11.15b
6	120	60	60	2.00	2.10a	29.08a	55.16a	26.38a	13.60a
7	120	75	60	1.60	2.11a	29.39a	55.28a	26.67a	13.79a
8	120	90	60	1.30	2.11a	29.51a	54.83a	27.15a	14.28a
9	120	120	60	1.00	2.11a	29.61a	55.54a	27.40a	14.48a
	CD (P=0.05)				0.25	2.04	4.38	3.22	1.69

For legend see Table 1

Leaf area and LAI

Nitrogen and phosphorus had a significant effect on the leaf area at all the growth stages (Table 3). The maximum leaf area was observed at the flowering stage. At the seedling stage, the leaf area did not differ between the treatments, except for the control, but the effect was significant at all other stages. Treatments involving N/P ratios of >1.0 or 1.0 with 120 kg N ha^{-1} produced greater leaf area than N/P ratios of <1.0 or the control in all stages except the seedling stage. The treatment with an N/P ratio of 1.0 and double rates of both N and P produced the largest leaf area in all the stages. At the flowering stage treatment with an N/P ratio of 1.0 or >1.0 with the higher dose of nitrogen (120 kg ha^{-1}) produced more leaf area (95.17 to 96.93 dm^2 $plant^{-1}$) as compared to other ratios (80.50 to 82.35 dm^2 $plant^{-1}$) and the control (65.83 dm^2 $plant^{-1}$). A similar trend was observed for LAI (Table 4). Higher LAI when nitrogen and phosphorus fertilizers were applied at an N/P ratio of >1.0 was also reported by Tomar et al. (1997). Higher LAI was due to the increased leaf area $plant^{-1}$, which was influenced by the higher leaf dry matter $plant^{-1}$ evidenced in the present investigation.

Correlation coefficients

The direct and indirect effects of photosynthetic attributes on the seed yield of sunflower can be estimated by separating the correlation coefficient (r value) and can be used to understand the exact relationship. In the present study, the number of green leaves $plant^{-1}$, dry matter accumulation in leaves, leaf area (dm^2 $plant^{-1}$) and leaf area index (LAI) were positively and significantly correlated (Table 5) with seed yield. All the photosynthetic attributes were highly significant at all the stages, which is clearly indicative of the positive relationship between photosynthetic attributes and the seed yield of sunflower.

Table 3

Leaf area (dm^2 $plant^{-1}$) of sunflower at different growth stages as influenced by nitrogen and phosphorus fertilization

No.	Treatments				Leaf area				
	N	P_2O_5	K_2O	N/P_2O_5	25 DAS	45 DAS	65 DAS	78 DAS	87 DAS
	(kg ha^{-1})								
1	0	0	60	–	5.00b*	51.66c	65.83c	33.66c	9.68c
2	60	75	60	0.80	7.33a	69.39b	80.50b	42.79b	14.76b
3	60	90	60	0.67	7.38a	64.82b	81.14b	42.84b	14.40b
4	90	75	60	1.20	7.57a	68.76b	80.64b	46.26b	15.12b
5	90	90	60	1.00	7.79a	69.07b	82.35b	46.03b	14.76b
6	120	60	60	2.00	7.74a	79.23a	95.51a	54.63a	18.81a
7	120	75	60	1.60	7.79a	78.93a	96.17a	54.38a	18.47a
8	120	90	60	1.30	7.74a	79..07a	96.54a	55.67a	19.40a
9	120	120	60	1.00	7.88a	79.31a	96.93a	55.68a	19.80a
	CD (P=0.05)				1.29	5.65	7.43	4.99	3.32

For legend see Table 1.

Table 4
Leaf area index (LAI) of sunflower at different growth stages as influenced by nitrogen and phosphorus fertilization

No.	Treatments N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	N/P_2O_5	LAI 25 DAS	LAI 45 DAS	LAI 65 DAS	LAI 78 DAS	LAI 87 DAS
1	0	0	60	–	0.320b*	2.693c	3.762c	2.020c	0.628c
2	60	75	60	0.80	0.480a	4.003b	4.890b	2.700b	0.820b
3	60	90	60	0.67	0.488a	4.073b	4.885b	2.830b	0.748bc
4	90	75	60	1.20	0.483a	4.163b	4.850b	2.833b	0.793bc
5	90	90	60	1.00	0.475a	4.008b	4.813b	2.867b	0.802bc
6	120	60	60	2.00	0.510a	4.413a	5.230a	3.200a	1.132a
7	120	75	60	1.60	0.513a	4.455a	5.225a	3.205a	1.225a
8	120	90	60	1.30	0.522a	4.503a	5.250a	3.138a	1.35a
9	120	120	60	1.00	0.530a	4.460a	5.273a	3.205a	1.148a
	CD (P=0.05)				0.098	0.283	0.217	0.217	0.171

For legend see Table 1.

Thus, the present investigation reveals that the application of nitrogen and phosphorus fertilizers at N/P ratios of 1.0 or > 1.0 with higher doses of nitrogen (120 kg N ha^{-1}) produced a higher number of green leaves plant^{-1}, more dry matter accumulation in the leaves, and more leaf area and LAI compared with N/P ratios of <1.0 and the control. The present study clearly indicated the effect of nitrogen on these photosynthetic attributes and its positive relationship with seed yield. The higher values of these characters led to the greater accumulation of photosynthates, which ultimately increased the seed yield of sunflower.

Table 5
Correlation coefficients (r values) between seed yield (kg ha^{-1}) and photosynthetic attributes of sunflower as influenced by nitrogen and phosphorus fertilization

No.	Parameter	r values
1	Number of green leaves at seedling stage	0.934**
2	Number of green leaves at button stage	0.891**
3	Number of green leaves at flowering stage	0.926**
4	Number of green leaves at seed formation stage	0.888**
5	Number of green leaves at maturity stage	0.911**
6	Leaf dry matter at seedling stage	0.965**
7	Leaf dry matter at button stage	0.926**
8	Leaf dry matter at flowering stage	0.897**
9	Leaf dry matter at seed formation stage	0.916**
10	Leaf dry matter at maturity stage	0.939**
11	Leaf area at seedling stage	0.941**
12	Leaf area at button stage	0.911**
13	Leaf area at flowering stage	0.920**
14	Leaf area at seed formation stage	0.950**
15	Leaf area at maturity stage	0.960**
16	Leaf area index at seedling stage	0.939**
17	Leaf area index at button stage	0.951**
18	Leaf area index at flowering stage	0.954**
19	Leaf area index at seed formation stage	0.967**
20	Leaf area index at maturity stage	0.940**

** Significant at the 5% level

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EFFECT OF PLANTING PATTERN AND N SPLITS ON YIELD ATTRIBUTES, YIELD AND QUALITY OF RAINFED SUNFLOWER (*HELIANTHUS ANNUUS* L.)

M. VIJAYAKUMAR, P. SUBBIAN, A. C. LOURDURAJ and R. SELVARAJU

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Field experiments were conducted at Tamil Nadu Agricultural University, Coimbatore, India during the North East Monsoon (October–December) seasons of 1997 and 1998 in a split plot design to study the effect of planting pattern and N splits on the yield attributes, yield and quality of rainfed sunflower. The main plot consisted of three plant populations (133,333 plants ha^{-1}, 111,111 plants ha^{-1} and 88,888 plants ha^{-1}) and the sub-plot treatments of six N split levels. The results revealed that the yield attributes of sunflower were higher at the closest spacing of 30 × 25 cm than at the widest spacing of 30 × 37.5 cm. The seed yield was higher at closer (30 × 25 cm) spacing in 1997 and at wider spacing (30 × 37.5 cm) in 1998. In both years the split application of nitrogen resulted in higher growth, yield attributes, seed yield and quality parameters when compared to full basal application.

Key words: sunflower, planting pattern, N splits, yield attributes, seed yield, quality

Introduction

Sunflower is an important oilseed crop in arid and semi-arid regions, ranking third in the world after soybean and groundnut in edible oil production. Being a photo-insensitive crop, sunflower has a wide range of adaptability to different agroclimatic conditions. However, under rainfed conditions where soil moisture is a limiting factor, maintaining an optimum plant population by adopting appropriate crop geometry is an important factor in increasing yields (Subba Reddy et al., 1997). Yield could be further increased by the application of fertilizer, particularly nitrogen (Tenebe et al., 1996). Here again the nitrogen needs to be applied in split doses in order to meet the requirements at critical stages of crop growth, which would promote the seed yield and in turn the oil yield of sunflower (Krishna Reddy et al., 1992). It was against this background that the field investigation was carried out under rainfed conditions to study the effect of planting pattern and split application of nitrogen on sunflower.

Materials and methods

Field experiments on sunflower were carried out at Tamil Nadu Agricultural University, Coimbatore, India during the Northeast Monsoon (October–December) seasons of 1997 and 1998. Coimbatore is situated in the Northwestern agroclimatic zone of Tamil Nadu at 11°N latitude and 77°E longitude and at an altitude of 426.7 m above mean sea level. The soil of the experimental field was a deep, moderately well-drained clay loam, low in available N (210 kg N ha^{-1}), medium

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in available P (17.5 kg P_2O_5 ha^{-1}) and high in available K (460 kg K_2O ha^{-1}). The electrical conductivity of the soil was 0.42 dS m^{-1} and the pH was 8.2. The field capacity, permanent wilting point and bulk density were 24.30%, 11.80% and 1.40 g cm^{-3}, respectively. Mechanical analysis of the soil showed 27.27%, 24.73%, 18.29% and 29.66% of coarse sand, fine sand, silt and clay, respectively. The sunflower variety CO.2, with a field duration of 87 days, was used in the trial. The dates of sowing and harvest were 18 Sept. and 30 Dec. in 1997 and 12 Sept. and 21 Dec. in 1998. The seeds were sown by dibbling 2 to 3 seeds $hill^{-1}$ in a flat bed with a row spacing of 30 cm and plant spacing as per the treatment schedule.

The experiments were laid out in a split plot design replicated three times. The treatment details were as follows:

I Main plot: Plant population
- P_1: 80% of recommended plant population (30 × 37.5 cm)
- P_2: 100% of recommended plant population (30 × 30 cm)
- P_3: 120% of recommended plant population (30 × 25 cm)

II Sub plot: Nitrogen split application
- N_1 : Full basal (40 kg N ha^{-1})
- N_2 : ½ basal + ½ in the 4th week after sowing (with rain)
- N_3 : ½ basal + ½ in the 6th week after sowing (with rain)
- N_4 : ½ basal + ¼ in the 4th week after sowing + ¼ in the 6th week after sowing (with rain)
- N_5 : ⅓ basal + ⅓ in the 4th week after sowing + ⅓ in the 6th week after sowing (with rain)
- N_6 : ¼ basal + ½ in the 4th week after sowing + ¼ in the 6th week after sowing (with rain)

The time of fertilizer application was fixed based on the simple water balance model results. Fertilizers were scheduled to be applied only when there was a greater chance of obtaining 15 mm or more rainfall during that particular week and when the soil moisture was sufficient. The treatments were imposed as planned, as 21.9 mm and 45.5 mm rainfall were received during the 4th and 6th week after sowing in 1997 and the corresponding mean soil available moisture contents were 49.5 mm and 47.1 mm, respectively. In 1998, 24.8 mm and 29.5 mm rainfall were received during the 4th and 6th week after sowing and the corresponding mean soil available moisture contents were 23.6 mm and 44.8 mm, respectively.

The recommended rate of N, P and K (40: 20: 20 kg ha^{-1}) was applied as urea (46% N), single superphosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. Nitrogen was applied in splits as per the treatment schedule. The full doses of P_2O_5 and K_2O were applied basally to all the treatments. The amount of rainfall received during the cropping period was 598 mm and 571.2 mm distributed over 43 and 20 rainy days during 1997 and 1998, respectively. The yield attributes and quality parameters recorded in the study were head diameter, total number of seeds $head^{-1}$, seed filling percentage, 100 seed weight, protein content and oil content. The diameter of five labelled flower heads per plot was measured at harvest and the mean was taken and expressed in cm. From that, the ratio of filled seeds to the total number of seeds $capitulum^{-1}$ was recorded and expressed as a percentage. The number of filled and unfilled seeds from the labelled heads was counted and recorded. One hundred filled seeds were counted from the heads of each plot and their weight was recorded in grammes. The seed yield from each net plot area was recorded at 14% moisture level and expressed in kg ha^{-1}. Seed samples were taken from each plot and analysed for total N by the micro-Kjeldahl method. The N content of the seed was multiplied by the factor 6.25 to obtain the crude protein content and expressed as a percentage. The oil content of the seeds was estimated using a Nuclear Magnetic Resonance (NMR) spectrometer (Bruter Minispe P_2O model) against a standard reference sample (Granlund and Zimmerman, 1975). The data collected were analysed statistically following the procedure given by Gomez and Gomez (1984). Wherever the treatment differences were significant, critical differences were calculated at the five per cent probability level. Since the study was conducted under rainfed conditions and the distribution of rainfall differed widely between the years (1997 and 1998), pooled analysis of data was not done and only the data for individual years are presented and discussed.

Results and discussion

Influence of plant population on yield, yield attributes and quality

Yield attributes: All the yield parameters were significantly higher under wider spacing (30 × 37.5 cm), because there was less below- and aboveground competition between the plants (Table 1). This led to greater light interception by the actively spreading, horizontally developing plants, which could thus reduce more atmospheric CO_2 into food materials because of greater initial light use efficiency. The reduction in the values of yield parameters at closer spacing is due to severe competition between the sunflower plants, especially under rainfed conditions where moisture is a major limiting factor throughout the crop growth period (Vijayalakshmi et al., 1975).

Seed yield: The sunflower seed yield was significantly influenced by the various spacings tested in both years. A closer spacing of 30 × 25 cm (133,333 plants ha^{-1}) gave a significantly higher yield than wider spacings of 30 × 30 cm and 30 × 37.5 cm in 1997. Though the yield attributes had higher values on a per plant basis under the widest plant spacing of 30 × 37.5 cm, the yield was higher under closer spacing due to the greater number of plants ha^{-1}. The percentage yield loss due to competition at higher density was compensated by the increased plant population per unit area. The higher values of yield components at greater plant density were the result of the better utilization of rainfall in a favourable environment and effective moisture utilization during high rainfall years (Reddy and Giri, 1997). In 1998, the increase in yield parameters at the lowest plant density of 88,888 plants ha^{-1} produced a higher seed yield on a per hectare basis. The spacing of 30 × 37.5 cm gave a significantly higher seed yield when compared to closer spacings of 30 × 30 and 30 × 25 cm. This may possibly be due to the severe competition between the plants under closer spacing, especially during the moisture stress experienced in the vegetative and early reproductive stages of the crop. Though the total rainfall received in the cropping period of 1998 (571 mm) was 78% higher than the normal seasonal rainfall of 321 mm, the distribution was not uniform, since there was a higher intensity of rainfall on a smaller number of rainy days. This situation led to a greater soil moisture deficit at higher plant population levels because of greater competition between the plants. It was also evident from other studies on sunflower that in relatively dry years an increased plant population cannot compete with the yields obtained at lower plant densities on a per plant basis (Dhoble et al., 1988). Rao and Reddy (1995) also reported the advantages of suboptimal plant populations under moisture stress conditions.

Seed quality: The widest spacing of 30 × 37.5 cm led to a significantly higher protein content compared with the 30 × 30 and 30 × 25 cm spacings in 1998. However, the effect was not significant in 1997 due to non-limiting moisture availability throughout the crop growth period. The oil content in the various spacing treatments did not differ greatly, though a slightly higher oil content was recorded at wider spacings. The higher protein and oil yields obtained at the widest spacing of 30 × 37.5 cm may be due to the higher seed filling percentage and test weight, which promoted the accumulation of more protein and oil (Kene et al., 1992).

Table 1
Effect of agrotechnical factors on yield attributes, yield and quality of rainfed sunflower

Treatments	Total No. of seeds		Head diameter (cm)		Filling percentage (%)		100 seed weight (g)		Seed yield (kg ha^{-1})		Protein content (%)		Oil content (%)	
	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
P_1	509.11	648.85	12.38	13.62	84.21	91.23	4.50	5.83	741	1022	17.67	18.53	37.05	36.70
P_2	473.22	651.74	11.52	12.52	82.25	85.61	4.45	5.59	757	953	17.63	18.52	37.17	36.74
P_3	446.05	583.81	11.15	11.70	79.36	77.40	4.43	5.08	810	838	17.62	18.47	37.18	36.79
SEd	15.36	4.83	0.14	0.06	0.37	0.21	0.03	0.05	8	4	0.21	0.11	0.09	0.07
CD P=(0.05)	42.65	13.43	0.38	0.17	1.03	0.59	NS	0.15	24	11	NS	0.23	NS	NS
N_1	451.33	625.93	11.01	12.66	81.26	85.15	4.32	5.56	730	938	17.03	18.35	37.23	36.8
N_2	476.22	657.46	11.33	13.71	82.47	88.27	4.44	5.88	762	998	17.25	18.79	37.13	36.2
N_3	455.22	599.71	11.23	11.55	80.98	80.94	4.52	5.02	746	883	17.34	18.07	37.12	37.5
N_4	501.11	625.33	12.30	11.97	82.73	84.23	4.56	5.44	812	932	18.36	18.57	37.01	36.2
N_5	499.22	615.77	12.09	12.45	82.47	83.21	4.40	5.40	774	911	17.08	18.29	32.20	37.2
N_6	473.56	644.58	12.15	13.34	81.72	86.67	4.52	5.72	792	964	18.07	18.96	37.10	36.1
SEd	16.66	6.03	0.14	0.21	0.58	0.14	0.02	0.15	21	6	0.11	0.02	0.16	0.90
CD P=(0.05)	34.02	12.31	0.27	0.42	1.19	0.29	0.05	0.11	42	13	0.22	0.04	NS	NS

NS: non significant

Influence of split application of nitrogen on yield, yield attributes and quality

Yield attributes: The yield attributes of sunflower were favourably influenced by the application of N in either two or three splits with rain in both the years compared to the full basal application. It was expected that when the nitrogen was applied in splits based on satisfactory soil moisture availability, the nutrient uptake would be increased substantially, thereby resulting in higher values of yield attributes. Increased yield attributes due to the split application of N in sunflower was also reported by Manoharan et al. (1991).

Seed yield: The split application of N resulted in a significant improvement in the seed yield of sunflower. The application of nitrogen in three splits (½ basal + ¼ in the 4th week after sowing + ¼ in the 6th week after sowing with rain) increased the seed yield by 11.2% over the full basal application of N. The higher seed yield obtained with split application of N was due to better growth and yield attributes of sunflower in the favourable monsoon seasons such as that of 1997, where there were fewer dry spells during the crop season. The better translocation of photosynthates to the reproductive parts was responsible for the improvement in the yield attributes and yield of sunflower (Reddy and Giri, 1997). However, in 1998 the application of nitrogen in two equal splits (½ basal + ½ in the 4th week after sowing with rain) resulted in a 9.53% greater seed yield compared with three equal splits of N. This may have been due to intermittent dry spells after the 4th week, which created a soil moisture deficit resulting in reduced nutrient uptake by sunflower at the active vegetative and flowering stages, leading to a reduced seed yield in the three-split N application.

Seed quality: In general, the split application of N favourably influenced the protein content of sunflower seed in both the years when compared to the full basal application of N. However, there was no marked difference between the N split applications with respect to oil content. The possible reason for the increased protein content in the three-split application of N may be the degradation of carbohydrates in the TCA (tricarboxylic acid) cycle, followed by further degradation to acetyl CO A (co-enzyme A) leading to more protein in the plant cells with an increased supply of nitrogen. Simultaneously, as the percentage of oil decreases, a very low amount of acetyl CO A is available for the synthesis of fatty acid under adequate available nitrogen (Bahl et al., 1997). Thus, it could be concluded from the results that when nitrogen is the limiting factor, the oil content could be higher. A similar inverse relationship between oil and protein content was reported by Loof (1960).

From the study, it is concluded that the highest plant population of 133,333 plants ha^{-1} combined with N in 3 splits (½ N basal + ¼ in the 4th week after sowing + ¼ in the 6th week after sowing with rain) was best for obtaining higher yields in sunflower under rainfed conditions with a high, uniform distribution of rainfall. However, when there is moisture stress in the vegetative and flowering stages due to the uneven distribution of rainfall, the lowest plant population of 88,888 plants ha^{-1} combined with recommended N in 2 splits (½ basal + ½ in the 4th week after sowing with rain) was found to be the best treatment for obtaining higher yields in rainfed sunflower.

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PROTEIN COMPOSITION IN DIFFERENT PHASES OBTAINED BY THE ULTRACENTRIFUGATION OF DOUGH

R. KUKTAITE[1], H. LARSSON[2] and E. JOHANSSON[1]

[1]DEPARTMENT OF CROP SCIENCE, THE SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES, ALNARP, SWEDEN; [2]DEPARTMENT OF FOOD TECHNOLOGY, LUND UNIVERSITY, LUND, SWEDEN

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Ultracentrifugation was used as a non-destructive method to separate dough into liquid, gel, gluten, starch and bottom phases. The protein composition in the different phases was investigated for dough prepared from spring wheat (*Triticum aestivum* L.). The SDS-PAGE, SE-HPLC and RP-HPLC methods were used for the analysis.

The wheat protein composition of the liquid and gel phases consisted of albumins, globulins and traces of gliadins and glutenins. The gluten phase contained proteins extractable with all the extraction buffers used. A similar protein composition was found in the starch and bottom phases, but in considerably lower amounts. Specific LMW glutenin subunits were identified in the gluten phase by RP-HPLC. The albumin composition differed in the gel phase compared to the gluten and bottom phases.

Differences in protein composition due to mixing methods were detected only for the albumin composition in the liquid phases.

Key words: dough mixing, ultracentrifugation of dough, dough phases, protein composition

Introduction

Bread-making quality is both an important and complex character of bread wheat (Pomeranz, 1988). Bread dough properties result from a balance and interaction between different components such as starch, gluten proteins, lipids, water, etc., where the protein fraction is the most important (Wall, 1979). The gluten proteins constitute the skeleton of the dough and give rise to the viscoelastic properties (Eliasson and Lundh, 1989; Eckert et al., 1994; Danno and Hoseney, 1982). The wheat flour components responsible for specific functional properties were fractionated and identified by Finney (1971). Recently, a new method has been developed to study dough properties: ultracentrifugation of the entire dough (separation into five phases: liquid, gel, gluten, starch and bottom) (Larsson and Eliasson, 1996 a;b). The amount of the separated phase and the water content of the individual phases were found to depend on wheat cultivars, dough water content (Larsson and Eliasson, 1996a), mixing time, added ascorbic acid, the presence of lipids (Larsson and Eliasson, 1996b), pH and salt (Larsson, 2002). Ultracentrifugation of the dough into different phases can be regarded as a non-destructive tool, which enables further investigation of the proteins present in their native form.

The aim of the present study was to investigate the protein composition of the different dough phases and how mixing methods affected the protein composition of these phases.

Materials and methods

Plant material

Flour of the cultivar Dragon, grown in 1996, was used to investigate the effect of the mixing process on the protein composition in the different phases. Furthermore, the protein composition of the different phases was investigated for six flours from spring wheat cultivars with different protein contents (10.0–12.3%, data not shown), grown in 1998.

Dough mixing

For the cultivar Dragon, grown in 1996, two different mixing methods were used, the farinograph method according to Larsson and Eliasson (1996 a;b) and the mixograph method (Reomixer, Bohlin Reologi, Öved, Sweden). For both methods, 10 g of flour was used and distilled water was added. An amount of water corresponding to the farinograph water absorption (44.8% total weight basis) was added to the dough mixed in the farinograph. For the mixograph method the quantity of water added to the dough was based on the flour protein content (46.9% total weight basis). Mixing was performed at 30°C for 6 minutes in the farinograph and 4.5 minutes in the mixograph.

For the six cultivars of flour from 1998, the mixograph method was used to produce dough with a water content ranging from 41.8% to 43.0% depending on the flour protein content.

Ultracentrifugation of dough

The dough was centrifuged (Finney, 1971) for 1 h at 100,000 g (25°C) in a Beckman Ultracentrifuge Optima LE80K (Beckman Instruments, USA). The dough sample (~ 12 g) was placed in a test tube with a diameter of 14 mm and a height of 89 mm. The water content of the different phases was determined according to Larsson and Eliasson (1996a).

SDS-PAGE

Wheat proteins were extracted from the freeze-dried dough phases prepared from Dragon flour grown in 1996. The extraction was performed in nine steps in the different ultra-centrifuged phases. The extraction buffers during the different steps were as follows:

(1) 0.6 ml H_2O,
(2) 0.6 ml 0.5M NaCl,
(3) 0.3 ml 0.5M NaCl,
(4) 0.6 ml 70% ethanol,
(5) 0.6 ml 50% 1-propanol,
(6) 0.3 ml 50% 1-propanol+1% dithiotreithol (DTT),
(7) 0.6 ml 50% 1-propanol+1% DTT+1% glacial acetic acid,
(8) 0.6 ml 0.5% sodium dodecyl sulphate (SDS)+1% DTT,
(9) 0.6 ml 6M urea+0.5% SDS+1% DTT.

The extracting material was 100 mg of ultra-centrifuged phases (gel, gluten and bottom). After each extraction, the supernatant was collected and a new buffer was added. The sample was suspended in the buffer, stirred for 30 min at 2,000 rpm and centrifuged for 30 min at 10,000 g to obtain the supernatant. Extractions (2) and (3) were performed at 4°C, (6) and (7) at 60°C and for extractions (8) and (9) the samples were heated to 100°C for 5 min. The other extractions were made at room temperature. The samples were rinsed with H_2O between extractions (3) and (4) in order to remove the salt. For the liquid and gel phases, no supernatants were formed during centrifugation after extraction (1). Thus, a higher amount of water was added in order to obtain a supernatant.

For the flour of six cultivars from 1998, wheat proteins from three of the ultra-centrifuged phases (gel, gluten and bottom) were extracted with 50% 1-propanol. All the samples were separated on 10% polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS). The gels were stained with Coomassie Brilliant Blue solution and destained according to Johansson (1996).

SE-HPLC

Proteins from three different phases (gel, gluten and bottom) of the six cultivars of flour from 1998 were fractionated through size-exclusion high performance liquid chromatography (SE-HPLC) (Johansson, 1996). Proteins from the different phases (11 mg) were extracted and separated on HPLC according to Johansson et al. (2001).

The chromatograms were divided into four sections with a decreasing molecular size range: large polymeric proteins (LPP, peak 1), smaller polymeric proteins (SPP, peak 2), large monomeric proteins (LMP, peak 3) and smaller monomeric proteins (SMP, peak 4) (Johansson et al., 2001; Kuktaite et al., 2000). The percentage of large unextractable polymeric protein in the total large polymeric protein (LUPP) was calculated as [peak 1 area (unextractable) / peak 1 area (total)] × 100. Peak 1 (total) refers to the total of peak 1 (extractable) and peak 1 (unextractable). In the same way, the percentage of total unextractable polymeric protein in the total polymeric protein (TUPP) was calculated as [peak 1+2 area (unextractable) / peak 1+2 area (total)] × 100. Peak 1+2 (total) refers to the total of peak 1+2 (extractable) and peak 1+2 (unextractable) (Johansson et al., 2001; Kuktaite et al., 2000).

RP-HPLC

Proteins from the gel, gluten and bottom phases were fractionated through reversed phase chromatography (RP-HPLC) (Kuktaite et al., 2000). The proteins were extracted in three steps in order to extract (1) albumins (Alb) and globulins (Glo), (2) gliadins (Gli) and (3) glutenin subunits (Glu) and separated on HPLC according to Johansson et al. (2001) and Weiser and Seilmeier (1998).

Statistical analysis and replicates

Spearman rank correlations and analyses of variance (ANOVA) were executed using the Statistical Analysis System (SAS User's Guide, 1985) program package. Two replicates of each sample were mixed and ultracentrifuged into different phases. SE-HPLC and RP-HPLC were done twice, except for the gel phase from RP-HPLC, which was analysed once.

Results

Ultracentrifugation

The most evident effect of the mixing procedure on the separation properties was that the bottom phase was smaller and the gluten phase was larger when the dough was mixed in the farinograph compared to the mixograph (Fig. 1). The mixing method did not influence the water content of the gluten phase (Table 1). The water content in the bottom and starch phases was smaller after mixing in the mixograph compared to the farinograph. The slightly larger water content observed in the liquid phase after separating dough mixed in the mixograph compared with the farinograph was consistent with the larger water content in the dough mixed in the mixograph (Table 1).

SDS-PAGE

Differences in protein composition were found between the phases. The liquid and gel phases contained mainly water- and salt-extractable proteins, i.e. albumins and globulins (Fig. 2A a-d). Only traces of gliadins and glutenins were found (Fig. 2A e, f; 2B a-f).

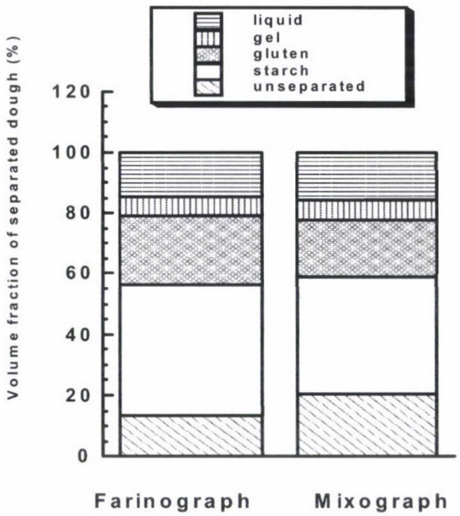

Fig. 1. Ultracentrifugation of dough mixed with farinograph (water content 44.8%) and mixograph (water content 46.9%).

Table 1

Water content in different phases obtained after ultracentrifugation of doughs mixed in the farinograph and mixograph. The water content of the dough differed for farinograph (44.8% wt) and mixograph (46.9% wt)

Dough phases	Farinograph water content, % wt	Mixograph water content, % wt
Liquid	87.0±0.6	89.0±0.4
Gel	83.2±0.3	83.2±0.5
Gluten	54.6±0.3	54.6±0.3
Starch	29.8±0.1	26.3±0.3
Bottom	33.0±0.6	27.1±0.1

The gluten phase mainly contained gliadins and glutenin subunits, but proteins extractable with all used extraction buffers were found (albumins, globulins, gliadins and glutenins) (Fig. 2C a-f). The protein compositions were similar for the gluten, starch and bottom phases (Fig. 2D, E a-f). The composition of water-extractable proteins in the liquid and gel phases was completely different from that in the gluten, starch and bottom phases.

The most prominent effect of the mixing method on the protein composition was observed for the water-extractable proteins in the liquid phase and only minor differences were found for the salt-extractable proteins, gliadins and glutenins (Fig. 2A c, d; 2A-E e, f).

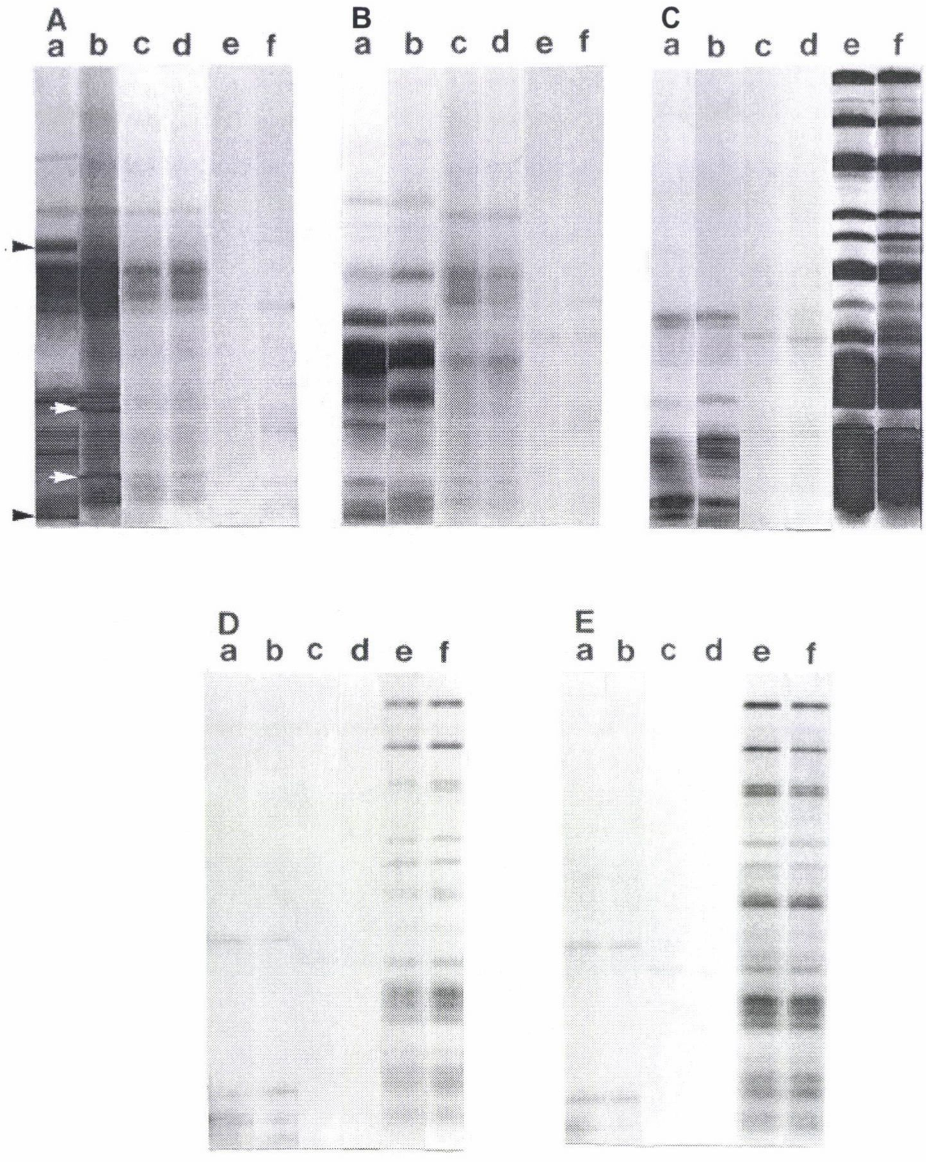

Fig. 2. SDS-PAGE of albumins (a, b - H_2O extraction), globulins (c, d - NaCl extraction), gliadins and glutenins (e, f - 6 M urea, 0.5% SDS, 1% DTT extraction) of different phases: A- liquid, B- gel, C- gluten, D- starch and E- bottom, after ultracentrifugation of dough. Different dough mixing methods were used: a, c, e - farinograph; b, d, f - mixograph

SE-HPLC

An example of the chromatograms obtained for the different phases of dough is shown in Fig. 3. Statistically significant differences between the three phases (gel, gluten and bottom) were observed for the relative amount of all monomeric and polymeric proteins (Table 2a). When the gluten phase was compared to the gel and bottom phases, Spearman rank correlation coefficients showed statistically significant, higher relative amounts of all monomeric and polymeric proteins except for SDS-soluble SMP. Higher relative amounts of SDS-soluble LPP and SPP, and of SDS-soluble and SDS-insoluble LMP and SMP, were found in the gel phase than in the bottom phase. The relative amounts of SDS-insoluble LPP and SPP, as well as the percentages of TUPP and LUPP were higher in the bottom phase than in the gel phase (Table 3a).

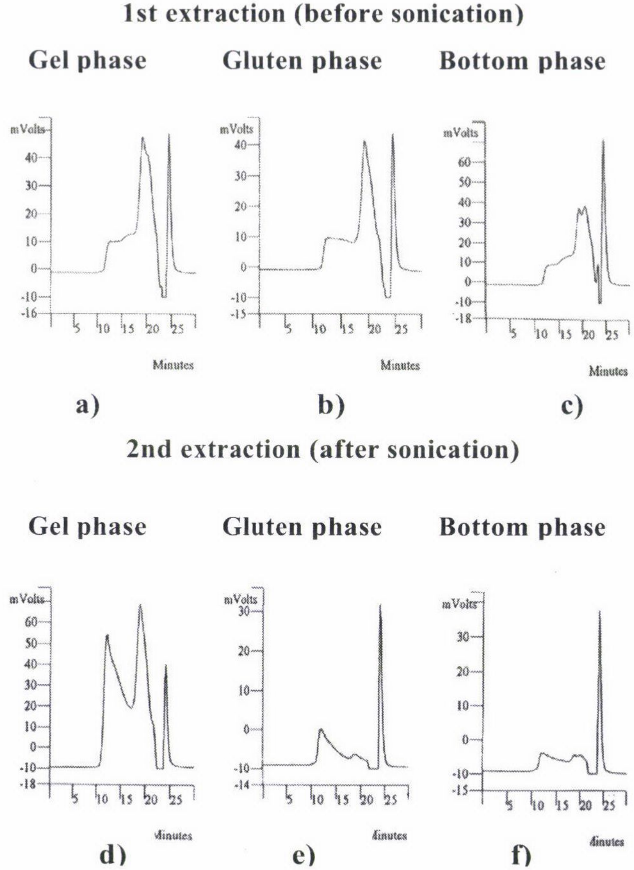

Fig. 3. SE-HPLC chromatograms of the different phases (gel, gluten and bottom) after ultracentrifugation of dough. 1st extraction - SDS-soluble monomeric and polymeric proteins; 2nd SDS-insoluble monomeric and polymeric proteins. In order to be able to analyse the samples extracted according to the method described for SE-HPLC, the samples were diluted: a) 3; b) 4; c) 0; d) 2; e) 3; f) 0 times

Table 2a

Mean squares from the analysis of variance of different protein parameters from SE-HPLC analyses

SDS-soluble	LPP (10^{13})	SPP (10^{14})	LMP (10^{14})	SMP (10^{14})	LUPP	TUPP
Phases	13.8***	6.4***	81.4***	1.9***	0.6***	0.7***
Error	0.1	0.5	0.3	0.0	0.0	0.0
SDS-insoluble	LPP (10^{14})	SPP (10^{15})	LMP (10^{14})	SMP (10^{13})	LUPP	TUPP
Phases	22.0***	3.3***	75.7***	2.0***	0.6***	0.7***
Error	0.5	0.0	0.3	0.0	0.0	0.0

LPP and SPP=large and smaller polymeric proteins, respectively, LMP and SMP=large and smaller monomeric proteins, respectively, TUPP=Total unextractable polymeric protein in the total polymeric protein, LUPP=Large unextractable polymeric protein in the total large polymeric protein

Table 2b

Mean squares from the analysis of variance of different protein parameters from RP-HPLC analyses

	Alb+Glo (10^{14})	Gli (10^{14})	Glu (10^{15})	Glu/Gli
Phases	388.6***	1589.0***	1603.3***	41.8***
Error	1.4	23.2	2.4	0.7

Alb+Glo=albumins+globulins, Gli=gliadins, Glu=glutenins, Glu/Gli=glutenin/gliadin ratio

Table 3a

Spearman rank correlation coefficients between the ultracentrifuged phases (gel, gluten and bottom) and protein parameters from SE-HPLC analyses

	Gluten-Gel	Gel-Bottom	Gluten-Bottom
SDS-soluble			
LPP	–0.83***	–0.69***	–0.87***
SPP	–0.71***	–0.57***	–0.87***
LMP	–0.38*	–0.83***	–0.87***
SMP	0.60***	–0.83***	–0.87***
SDS-insoluble			
LPP	–0.87***	0.56***	–0.86***
SPP	–0.87***	0.17	–0.87***
LMP	–0.87***	–0.87***	–0.87***
SMP	–0.87***	–0.87***	–0.87***
LUPP	–0.81***	0.67***	–0.63***
TUPP	–0.83***	0.74***	–0.87***

Table 3b

Spearman rank correlation coefficients between the ultracentrifuged phases (gel, gluten and bottom) and protein parameters from RP-HPLC analyses

	Gluten-Gel	Gel-Bottom	Gluten-Bottom
Alb+Glo	–0.76***	–0.74***	–0.87***
Gli	–0.82***	0.82***	–0.87***
Glu	–0.82***	0.78***	–0.87***
Glu/Gli	–0.74***	–0.54***	–0.84***

For abbreviations see Table 2.

RP-HPLC

RP-HPLC analyses showed differences in protein composition between the different phases (Fig. 4). The gel phase contained mainly albumins and globulins and only traces of gliadins and glutenins (Fig. 4 a, d, g). The gluten and bottom phases contained mainly gliadins and glutenins, with some albumins and globulins (Fig. 4 b, c, e, f). The protein composition in the gluten and bottom phases was similar except for differences in the low molecular weight-glutenin subunit (LMW-GS) composition (Fig. 4 h, i, marked by arrows). The albumin and globulin compositions differed greatly between the gel phase and the gluten and bottom phases, respectively (Fig. 4 a-c).

ANOVA analyses showed statistically significant differences between the total values of different protein groups in the phases (Table 2b). Spearman rank correlations showed higher values of all types of proteins in the gluten phase than in the gel and bottom phases (Table 3b). A higher amount of albumins and globulins (Alb+Glo) and a higher ratio of glutenins to gliadins (Glu/Gli) were found in the gel than in the bottom phase. The Glu/Gli ratio was higher in the bottom phase than in the gel phase (Table 3b).

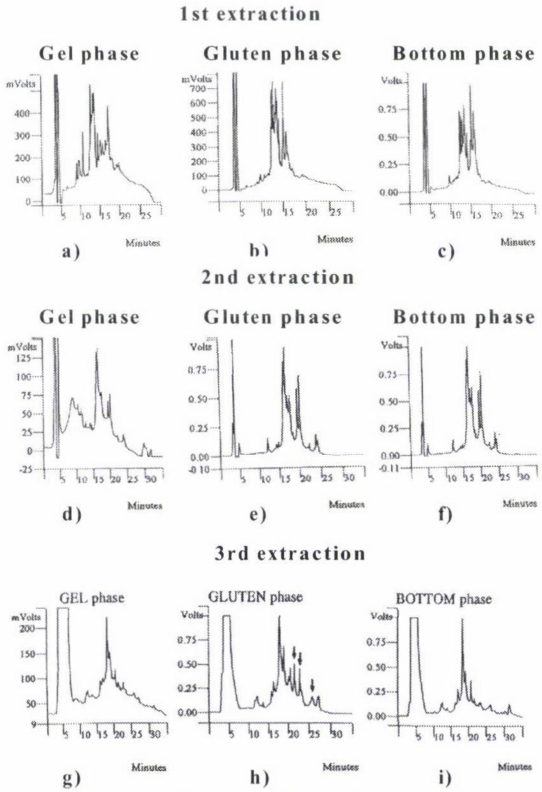

Fig. 4. RP-HPLC chromatograms of the different phases (gel, gluten and bottom) after ultracentrifugation of dough. 1st extraction - albumins and globulins; 2nd - gliadins, 3rd - glutenins. In order to be able to analyse the sample extracted according to the method described for RP-HPLC, the samples were diluted: a) 4; b) 8; c) 0; d) 0; e) 12; f) 4; g) 0; h) 24; i) 2 times

Discussion

Most of the HMW-GS and LMW-GS found in the gluten phase were present in the bottom phase, although the relative amounts were higher in the gluten phase than in the bottom phase. However, specific LMW-GS were found only in the gluten phase and not in the other phases. These specific LMW-GS were found in the gluten phase in all flours and under all mixing conditions, and may be of importance for gluten formation and bread-making quality.

In addition to the albumins and globulins found in the gel phase, traces of gliadins and glutenins were also detected. This material may contain proteins associated with the starch granule surface (Larsson and Eliasson, 1996a). Certain proteins have also been identified as associated with water-washed wheat starch (Greenwell and Schofield, 1986; Eliasson and Tjerneld, 1990).

Water- and salt-soluble proteins, i.e. albumins and globulins, were found not only in the liquid and gel phases, but also in the gluten and bottom phases. This is in accordance with earlier findings (Finney, 1971; Gupta et al., 1991; Figueroa and Khan, 1993) on the albumins and globulins found in gluten. However, the composition of water- and salt-soluble proteins differed between the gel phase and the gluten and bottom phases in this study. The differences were found in all investigated flours. The proteins found in the liquid and gel phases are not thought to contribute notably to the formation of the gluten network. According to Finney (1971), albumins and globulins are trapped in gluten during its formation. However, the specific albumins and globulins found in the gluten phase in this study were different to those found in the liquid and gel phases.

The most obvious difference in the mixing action between the two methods is that shearing of the dough dominates in the farinograph, whereas in the mixograph the dough is stretched and folded between the mixograph pins.

However, the different mechanical treatments did not produce changes in water content or in the protein composition of the gluten phase.

The main differences in the protein composition due to the mixing method were found in the liquid phase.

This may have been caused by the fact that dough prepared in the mixograph contained more water than that mixed in the farinograph. However, the amount of the liquid phase recovered was approximately the same for the two mixing methods. The water content of the liquid phase was slightly larger for dough mixed in the mixograph (Table 1). Thus, the effect on the water-soluble proteins may be related to differences in water content in the liquid phases. In conclusion, the effect of mixer type can be considered small compared with, for example, the influence of mixing time (Larsson and Eliasson, 1996b).

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SPLIT STRATEGIES AND INTERCROPPING DHAINCHA FOR *IN SITU* GREEN MANURING AND SEED PRODUCTION IN WET-SEEDED RICE

S. ANBUMANI[1], B. CHANDRASEKARAN[2], T. NADANASSABABADY[3] and P. RAJENDRAN[2]

[1]CENTRAL INSTITUTE FOR COTTON RESEARCH, COIMBATORE, INDIA
[2]TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA
[3]SOIL AND WATER MANAGEMENT RESEARCH INSTITUTE, THANJAVUR, INDIA

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In a wet-seeded rice establishment system, it is feasible to raise dhaincha (*Sesbania aculeata.* W.) in alternate rows as an intercrop using a newly developed joint rice and green manure seeder. Intercropping dhaincha exclusively for *in situ* green manuring recorded a higher grain yield of rice (with green manure) than sole rice. Besides *in situ* green manuring, leaving every 20th row for seed production achieved not only the self manuring of the land, but also the self-production of green manure seed (dhaincha). Leaving dhaincha at narrow spacings (10th or 15th row) for seed production affected the growth, development and yield of rice in the adjacent rows. Within the total dose of fertiliser N, split dose of 25, 33, 21 and 21% at 20 days after sowing (DAS), at green manure incorporation (37 DAS), 55 DAS and 70 DAS were found to be the ideal method of N application, alleviating the temporary lock-up of N, if any, upon green manure incorporation. Thus, the sustainability of the green manure intercrop for *in situ* incorporation in the rice culture and of green manure seed production were proved by the study.

Key words: wet-seeded rice, dhaincha, intercropping

Introduction

Rice is a staple food crop in India and it continues to hold the key to sustainable food production by contributing 20–25% of the agricultural gross domestic product (GDP) and to assuring food security in India for more than half of the total population. However, from the standpoint of net returns to the farmers, it is a lacklustre crop. The declining trend in rice productivity under irrigated ecosystems is a matter of paramount concern. Intensive cultivation has been shown to potentially degrade the resource base (Pingali et al., 1990). Hence, soil research emphasizes the prevention of soil health deterioration and yield decline. The declining trend is mainly attributed to the reduced use of organic manures and to micronutrient deficiencies (Ramalingam et al., 2000). Green manures constitute a feasible and appropriate substitute for building up soil fertility without endangering the resource base. However, in an intensive, profit-oriented cropping system, the situation may not permit farmers to utilise their already small land area for 6 to 7 weeks exclusively for green manure cultivation without any revenue (Kannaiyan, 2000). This apart, the separate cultivation of green manure prior to or after rice requires tillage and seeding operations which involve labour and cash outlays that may be oppressive for farmers (Garrity and Flinn, 1988).

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Therefore, evolving a suitable system for fitting in green manures without sacrificing any of the economic crops in the system mooted the strategy of growing green manures along with rice as a dual culture. There is a greater possibility of intercropping green manures during the early stage of the rice crop when they cause less interference with rice growth (Mathew et al., 1991). This situation can effectively be capitalised on by raising dhaincha as a green manure crop concurrently with wet-seeded rice using the Tamil Nadu Agricultural University (TNAU) rice and green manure seeder developed by Rajendran et al. (1999). *Sesbania aculeata* W. is a green manure crop widely cultivated in India. It has wide adaptability (water logging, salinity, severe drought) and accumulates large biomass in a short period of time (Pandey, 1996).

Availability of seed is a major constraint for the large-scale adoption of green manures in integrated nutrient management, even though the use of green manures is an age-old practice. Scarcity of seed is the main impediment to the quick expansion of *Sesbania* green manuring. There is also little scope for growing *Sesbania* as a sole crop for seed production, as most of the land is harnessed for rice cultivation in the lowland ecosystem (Sirajul Islam et al., 1999). The present study was undertaken to overcome the above constraints, keeping in view that the rice land itself should produce rice, green manure and green manure seed for sowing in the succeeding season.

Materials and methods

Field experiments were conducted during the dry (June–September) and wet (October-January) seasons of 1999 and the dry season of 2000 on the wetland farm of TNAU in Coimbatore (11° N; 77° E; 426 masl). The experimental site received a mean annual rainfall of 674.2 mm on 49 rainy days with mean maximum and minimum temperatures of 31.5°C and 21°C, respectively. The soil of the experimental field was clay loam in texture (Typic Haplustalf) and was low in available nitrogen, medium in available phosphorus and high in available potassium. The experiment was conducted in a split-plot design with three replications. Intercropping treatments were assigned to the main plots, while split doses of N made up the subplots. Besides the exclusive green manuring treatment, every 10th, 15th or 20th row was left for seed production with a check plot of sole rice for comparison. The nitrogen split doses tested were 25:25:25:25, 25:33:21:21 and 25:42:16.5:16.5% at 20, 37, 55 and 70 days after sowing (DAS), respectively. The rice and green manure were sown using a manually operated "single wheel rice *cum* green manure seeder" developed by TNAU. The seeder has a single central wheel and is designed to sow rice and green manure (dhaincha) in alternate rows. The seeder width is 0.75 m and the walking speed of the operator is around 1.5 km h^{-1}. Two men will cover 0.54 ha day^{-1}.

The short duration (115 days) rice cultivar ADT 43 and the medium duration (135 days) rice cultivar ADT 38 were sown during the dry (June–October) and wet (October–March) seasons, respectively, at a seeding rate of 80 kg ha^{-1}. Dhaincha, a leguminous green manure (GM) crop, was sown as intercrop at 30 kg ha^{-1}. Dhaincha is an indigenous green manure crop familiar to Indian farmers. It is traditionally grown as a root nodulating, quick growing, succulent green manure, capable of producing 20 to 25 tonnes of biomass ha^{-1}, accumulating 80–120 kg N ha^{-1} in 55 days. It is tolerant to flooding and salinity and does not require seed scarification to increase seed germination.

The intercropped dhaincha intended for green manuring was incorporated *in situ* at 37 DAS using an IRRI 'Cono weeder'. Dhaincha rows intended for seed production were retained as such for every 10^{th}, 15^{th} and 20^{th} row, as per treatments. The recommended doses of 120:38:38 kg ha^{-1} (dry season) and 150:50:50 kg ha^{-1} (wet season) of nitrogen, phosphorus and potassium were applied in the form of urea (46% N), single superphosphate (16% P_2O_5) and muriate of potash (60% K_2O). The split application of N was done as per treatments and the entire dose of phosphorus was applied basally before sowing. Potassium was applied in equal quantities at 20, 40 and 55 DAS. Rice and dhaincha samples were collected from a pre-designated sampling area on either side of the plot excluding two border rows.

Results and discussion

Dhaincha growth parameters and N contribution

Among the rice + dhaincha intercropping treatments (I_1 to I_4) the dhaincha population m^{-2} was marginally higher during the wet season than in the dry season, but within each season the population was similar.

The quantity of biomass added to the soil (Table 1) differed greatly between the intercropping treatments because a number of rows of dhaincha were retained for seed production after *in situ* incorporation. More biomass was added where dhaincha was grown purely for green manuring purposes (I_1), being 6.13, 5.59 and 6.65% higher than when every 20^{th} row of dhaincha was retained for seed production and 15.33, 18.41 and 16.14% higher than when every 10^{th} row was retained for seed production in the dry and wet seasons of 1999 and the dry season of 2000, respectively. Nitrogen split application did not have any impact on dhaincha biomass production since the nitrogen management treatments were imposed only after the *in situ* incorporation (37 DAS) of intercropped dhaincha.

The intercrop of dhaincha was incorporated *in situ* (37 DAS) in all seasons. The nitrogen content of the dhaincha at the time of incorporation was 2.57–2.70% in all seasons. N accumulation also varied greatly with the quantity of biomass produced. Dhaincha grown exclusively for green manuring (I_1) produced more biomass resulting in a greater accumulation of N. This was followed by the treatment in which every 20^{th} row was left for seed production (I_4). In comparison with the treatment leaving every 10^{th} row for seed production (I_2), 11.9 to 13.1 kg N ha^{-1} higher N accumulation was observed when utilizing dhaincha entirely for green manuring (I_1). Retaining a few rows (10:1, 15:1 and 20:1) for seed production resulted in a reduction in both biomass addition and N contribution in all the seasons.

Rice growth parameters

Rice plant height was highest when dhaincha was grown for green manuring alone (I_1) and was comparable with rice + dhaincha intercropping when every 20^{th} or 15^{th} dhaincha row was left for seed production (I_4 and I_3). The lowest plant height was observed in sole rice (I_0). The split application of N was found to increase plant height in four equal applications (N_1). However, it was comparable with N_2, where 33% of the N was applied at 37 DAS (Table 1).

Table 1

Growth parameters of dhaincha and rice as affected by green manure intercropping and N application

Treatments	Dhaincha			Rice		
	1[+]	2	3	4	5	6
Dry season (June–October), 1999						
Rice + green manure intercropping						
Sole wet-seeded rice (I_0)	–	–	–	77.9	479	4.31
Dhaincha entirely for GM (I_1)	73.5	14.4	77.5	84.7	517	4.76
10^{th} row for SP and others for GM (I_2)	74.3	12.2	65.6	81.3	498	4.51
15^{th} row for SP and others for GM (I_3)	75.1	12.9	69.9	82.8	504	4.60
20^{th} row for SP and others for GM (I_4)	74.7	13.5	72.7	83.5	510	4.68
CD (P=0.05)	NS	0.38	2.40	3.37	17.1	0.19
N split doses*						
25:25:25:25 (N_1)	73.8	13.3	72.0	84.0	492	4.47
25:33:21:21 (N_2)	74.4	13.1	70.6	83.1	500	4.57
25:42:16.5:16.5 (N_3)	75.0	13.3	71.7	79.0	513	4.68
CD (P=0.05)	NS	NS	NS	4.03	11.9	0.20
Wet season (October–January), 1999						
Rice + green manure intercropping						
Sole wet-seeded rice (I_0)	–	–	–	85.8	557	4.96
Dhaincha entirely for GM (I_1)	82.1	12.9	65.6	92.4	603	5.37
10^{th} row for SP and others for GM (I_2)	83.6	10.5	53.6	89.5	572	5.11
15^{th} row for SP and others for GM (I_3)	81.8	11.5	58.6	90.7	584	5.21
20^{th} row for SP and others for GM (I_4)	83.0	12.2	63.1	91.8	592	5.30
CD (P=0.05)	NS	0.33	2.70	2.93	14.3	0.16
N split doses*						
25:25:25:25 (N_1)	81.8	11.6	60.3	92.3	574	5.07
25:33:21:21 (N_2)	82.6	12.0	59.0	90.8	579	5.19
25:42:16.5:16.5 (N_3)	83.5	11.7	61.4	87.0	592	5.31
CD (P=0.05)	NS	NS	NS	2.21	12.3	0.22
Dry season (June–October), 2000						
Rice + green manure intercropping						
Sole wet-seeded rice (I_0)	–	–	–	80.1	501	4.80
Dhaincha entirely for GM (I_1)	69.5	15.8	81.2	86.6	539	5.23
10^{th} row for SP and others for GM (I_2)	68.1	13.3	68.1	83.9	519	4.89
15^{th} row for SP and others for GM (I_3)	67.8	14.2	72.7	85.1	526	5.05
20^{th} row for SP and others for GM (I_4)	68.6	14.7	75.8	86.0	531	5.13
CD (P=0.05)	NS	0.41	3.00	2.68	16.8	0.19
N split doses*						
25:25:25:25 (N_1)	67.5	14.3	73.4	86.3	516	4.91
25:33:21:21 (N_2)	68.4	14.5	74.3	85.1	522	5.01
25:42:16.5:16.5 (N_3)	69.6	14.8	75.7	81.6	532	5.13
CD (P=0.05)	NS	NS	NS	3.02	9.8	0.18

[+]1: Population (m^{-2}), 2: Biomass (t ha^{-1}), 3: Nitrogen accumulation (kg ha^{-1}), 4: Plant height at maturity (cm), 5: No. of tillers at flowering (m^2), 6: Leaf area index at flowering; GM: Green manuring; SP: Seed production; *N split doses were applied at 20, 37, 55 and 70 days after sowing

The largest number of tillers was recorded when rice was intercropped with dhaincha entirely for green manuring purposes (I_1) and the smallest number of tillers in sole rice (I_0). The treatment where dhaincha was grown entirely for green manuring (I_1) purposes was on par with those where every 20th or 15th dhaincha row was left for seed production and the remaining rows used for green manuring (I_4 and I_3) during the dry seasons of 1999 and 2000. In the 1999 wet season, dhaincha grown entirely for green manuring was on par with I_4.

The leaf area index at the flowering stage was significantly enhanced by the dhaincha intercropping treatments compared to sole rice, irrespective of the season. Sole rice (I_0) registered the lowest LAI, but this was on par with leaving every 10th dhaincha row for seed production and the remaining rows for green manuring (I_2) in the 1999 dry and wet seasons. During the 2000 dry season, however, it resulted in lower LAI than I_2. Split applications of N had a significant influence on LAI. The application of 42% N at the time of dhaincha incorporation (N_3) gave higher LAI in all seasons than four equal splits of N (N_1) and was comparable with N_2 in all the seasons. An increased N supply at the vegetative stage of the crop produced taller plants with profuse tillering and enlarged leaf size, as reported by Muralidharan (1996).

Rice grain yield

Growing dhaincha entirely for green manuring (I_1) was better than the other treatments in terms of grain yield (Table 2). Leaving every 20th dhaincha row for seed production and using the remaining rows for green manuring (I_4) was found next best. In the wet season, leaving every 20th (I_4) or 15th (I_3) row of dhaincha for seed production after the incorporation of the remaining dhaincha for green manuring produced similar grain yields. Intercropping *Sesbania* in wet-seeded rice added higher biomass, thereby supplementing the N needs of the rice crop and inducing a synergistic effect between the organic source of N and fertiliser N. This phenomenon maximised the grain yield in green-manured plots (Bayan, 2000).

The split application of N had an augmenting effect on the grain yield irrespective of the season. The application of 33% N at the time of dhaincha incorporation (N_2) led to significantly higher grain yield than four equal N splits (N_1). Nitrogen is known to improve grain production, since it is a prime substrate for the synthesis of the organic N compounds which constitute protoplasm and chloroplasts (Beringer, 1980). The split application of 33% at dhaincha incorporation (N_2) and four equal splits (N_1) performed equally well for grain yield except in the 1999 dry season. This could be attributed to the fact that N top dressing at the panicle initiation stage enhanced the transfer of assimilates from the flag leaf during the ripening phase (Wang and Zhang, 1995). The interaction effect was significant between dhaincha intercropping and N split application in all seasons. The best treatment combination in terms of higher grain yield was growing dhaincha entirely for green manuring along with 33% N application during dhaincha incorporation (I_1 N_2). Retaining every 20th row of dhaincha for seed production along with 33% N application at the time of dhaincha incorporation (I_4 N_2) was found to be the next best combination.

Table 2

Grain and straw yield of rice and dhaincha seed yield as influenced by dual cropping and N application

Treatments	Grain yield (t ha^{-1})				Dhaincha seed yield (kg ha^{-1})			
	N_1	N_2	N_3	Mean	N_1	N_2	N_3	Mean
Dry season (June–October), 1999								
Rice + green manure intercropping								
Sole wet-seeded rice (I_0)	5.34	5.04	4.62	5.03	–	–	–	–
Dhaincha entirely for GM (I_1)	6.31	6.76	5.82	6.00	–	–	–	–
10^{th} row for SP and others for GM (I_2)	5.37	5.76	4.96	5.36	173	171	166	170
15^{th} row for SP and others for GM (I_3)	5.71	6.13	5.27	5.70	134	138	139	137
20^{th} row for SP and others for GM (I_4)	5.99	6.42	5.53	5.98	105	110	112	109
Mean	5.76	6.02	5.24	–	136	139	141	–
CD (P=0.05)								
I		0.132				**		
N		0.163				**		
I at N		0.250				**		
N at I		0.360				**		
Wet season (October–January), 1999								
Rice + green manure intercropping								
Sole wet-seeded rice (I_0)	4.65	4.31	3.95	4.30	–	–	–	–
Dhaincha entirely for GM (I_1)	5.44	5.72	5.01	5.30	–	–	–	–
10^{th} row for SP and others for GM (I_2)	4.61	4.84	4.24	4.56	127	132	137	183
15^{th} row for SP and others for GM (I_3)	4.88	5.13	4.49	4.84	94	96	101	145
20^{th} row for SP and others for GM (I_4)	5.19	5.46	4.75	5.14	73	75	74	117
Mean	4.95	5.09	4.49	–	98	101	104	–
CD (P=0.05)								
I		0.163				**		
N		0.143				**		
I at N		0.308				**		
N at I		0.312				**		
Dry season (June–October), 2000								
Rice + green manure intercropping								
Sole wet-seeded rice (I_0)	5.71	5.35	4.99	5.35	–	–		
Dhaincha entirely for GM (I_1)	6.58	6.81	6.01	6.47	–	–		
10^{th} row for SP and others for GM (I_2)	5.52	5.71	5.04	5.43	179	184	186	183
15^{th} row for SP and others for GM (I_3)	5.86	6.09	5.37	5.78	148	144	148	148
20^{th} row for SP and others for GM (I_4)	6.36	6.58	5.80	6.25	116	116	119	119
Mean	6.01	6.11	5.44	–	146	148	151	117
CD (P=0.05)								
I		0.159				**		
N		0.176				**		
I at N		0.301				**		
N at I		0.387				**		

GM: Green manuring; SP: Seed production; N_1: 25:25:25:25; N_2: 25:33:21:21; N_3: 25:42:16.5:16.5; *N split doses were applied at 20, 37, 55 and 70 days after sowing; **Data not analysed statistically

Dhaincha seed yield

Retaining every 10^{th} row of dhaincha for seed production and using the remaining rows for green manuring (I_2) gave a higher dhaincha seed yield than leaving every 15^{th} or 20^{th} dhaincha row for seed production. The split application of N, with 42% at the time of dhaincha incorporation (N_3), gave the highest seed yield, but the difference in yield due to the treatments was negligible. The season had a considerable influence on dhaincha seed yield, with higher seed yields being recorded during the dry season compared to the wet season.

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EFFECT OF INTEGRATED MANAGEMENT OF IRRIGATION, COMPOSTED COIR PITH AND NUTRIENTS ON THE GROWTH AND YIELD OF SOYBEAN (*Glycine max* L. Merr.)

N. K. PRABHAKARAN and A. C. LOURDURAJ

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Field experiments were conducted during the summer (February–May) and south west monsoon (June–September) seasons of 1996 and 1997 at the Aliyarnagar Agricultural Research Station of Tamil Nadu Agricultural University, India, to study the growth and yield of soybean in response to irrigation, composted coir pith, time of N, P, K application and use of a nutrient mixture spray. The results revealed that irrigation at 0.90 IW/CPE [ratio of Irrigation Water Depth (IW) to Cumulative Pan Evaporation (CPE)], the application of composted coir pith and the split application of N, P and K in conjunction with a nutrient mixture spray significantly increased the plant height, leaf area index, dry matter production and grain yield of soybean. However, the root length of soybean was significantly reduced by irrigating at 0.90 IW/CPE (compared to irrigation at 0.70 IW/CPE and 0.50 IW/CPE) and by the application of composted coir pith.

Key words: soybean, irrigation, nutrients, growth, yield

Introduction

Globally soybean is cultivated on an area of 55.8 million hectares with a production of 102.2 million tonnes (Rammohan et al., 1992). One of the constraints responsible for the low productivity of soybean is lack of sufficient water for irrigation. Therefore, irrigation management in soybean is quintessential for improved efficiency, higher productivity, better economic returns and sustained soil health. A climatological approach has been used for scheduling irrigation based on irrigation water depth and cumulative pan evaporation. The assessment of water needs based on day-to-day weather parameters seems to be more rational than any other method (Senthilkumar, 1990). Various IW/CPE ratios have been reported to give better growth and yield in soybean. Purushothaman et al. (1992) obtained the highest yield with an IW/CPE ratio of 0.75 and Patel and Patel (1995) with a ratio of 1.0.

Though we can optimize water use, success depends on the efficient utilization of the water received through irrigation during crop growth. The addition of coir pith (coconut coir rope factory waste), besides increasing the water-holding capacity, brings about favourable changes in drainage, mulching, crop rooting, soil reconditioning and seed germination (Ravindranath, 1991). It is assessed that in India 7.5 million tonnes of coir pith is produced annually (Kamaraj, 1994). There is a dearth of information on the efficiency of composted coir pith in improving the utilization of applied water in soybean.

Numerous attempts have been made to quantify the nutrient requirements of soybean, but less attention has been paid to the timing of nutrient application. Another method for increasing fertilizer use efficiency is foliar nutrition, which reduces the loss of nutrients through leaching or due to unfavourable soil conditions. A number of research reports are available on the beneficial effects of foliar nutrition on the growth and yield of soybean (Hegazy et al., 1990; Srinivasan and Ramaswamy, 1992).

In view of the above facts, the present study was undertaken to investigate the effect of different irrigation regimes, composted coir pith, time of N, P, K application and use of a nutrient mixture spray on the growth and yield of soybean.

Materials and methods

Field experiments were conducted in soybean during the summer (February–May) and south west monsoon (June–September) seasons of 1996 and 1997 at the Aliyarnagar Agricultural Research Station of Tamil Nadu Agricultural University, India, geographically situated at 10°39' N latitude and 77°0' E longitude at an altitude of 260 m above mean sea level.

The soil of the experimental field was well-drained sandy clay loam with a pH of 7.4, EC of 0.4 dS m^{-1}, organic carbon content of 0.33%, low available N (216 kg ha^{-1}), medium available P (17.6 kg ha^{-1}) and high available K (281 kg ha^{-1}). The bulk density of the soil was 1.41 Mg m^{-3} with a field water capacity of 23.15% and a permanent wilting point of 12.5%. The experiment was laid out in a split plot design with three replications.

The treatment details were as follows:

Main plot treatments

1. Water use factors:

I_1 – Irrigation at 0.50 IW/CPE ratio [ratio of irrigation water depth (IW) to cumulative pan evaporation (CPE)]

I_2 – Irrigation at 0.70 IW/CPE ratio

I_3 – Irrigation at 0.90 IW/CPE ratio

2. Composted coir pith (CCP) levels:

C_1 – Control (without composted coir pith)

C_2 – Composted coir pith @12.5 t ha^{-1}.

Sub-plot treatments

Fertilizer management practices:

F_1 – Recommended dose of NPK – all basal

F_2 – Recommended dose of NPK in two splits [50% as basal + 50% as top dressing at 30 days after sowing (DAS)]

F_3 – F_1 + nutrient mixture spray (2% concentration) twice at 30 and 45 DAS

F_4 – F_2 + nutrient mixture spray (2% concentration) twice at 30 and 45 DAS.

The fertilizer schedule recommended for soybean in Tamil Nadu (20:80:40 kg NPK ha^{-1}) was adopted. The nutrients were applied in the form of urea (46% N), single super-phosphate (16% P_2O_5) and muriate of potash (60% K_2O) as per the treatment schedule. The nutrient mixture consisted of di-ammonium phosphate (1.0%), MOP (0.50%) and a micronutrient mixture (0.5%), giving a total concentration of 2%. Iron (4.0%), zinc (3.0%), manganese (2.0%) and copper (0.1%) were included in the micronutrient mixture. The nutrient mixture spray solution was prepared (at the rate of 500 litres ha^{-1}) the previous day, and the supernatant solution alone was used for spraying with a hand-operated knapsack sprayer.

The experimental field was ploughed and harrowed, and flat beds were formed. A gross plot size of 5.0 m × 4.2 m and a net plot size of 4.8 m × 3.6 m was adopted. Composted coir pith produced from raw coir pith using the methodology of Nagarajan et al. (1987) was incorporated basally in the respective plots after forming flat beds but before levelling the field. The composted coir pith had a pH of 7.10, EC of 0.36 dS m^{-1}, C:N ratio of 25:1 and N, P and K contents of 0.99, 0.07 and 1.09%, respectively. Seeds of soybean variety CO.1, which is photo-insensitive, of determinate type, erect in habit and matures in 85–90 days, were dibbled in lines, with a row spacing of 30 cm and a plant to plant spacing of 5 cm.

For scheduling irrigation based on the climatological approach, the evaporation rate from a USWB class A open pan evaporimeter was recorded daily. A common depth of irrigation, i.e. 60 mm, was adopted. Thus, irrigation was given to 0.50 (I_1), 0.70 (I_2) and 0.90 (I_3) IW/CPE ratios, whenever cumulative pan evaporation reached the level of 120, 85.7 and 66.7 mm, respectively. A Parshall flame with 7.5 cm throat width was used for measuring the volume of water before letting it into each plot.

Five plants selected at random from the net area of each plot were tagged and the growth characters were recorded at 60 DAS. For recording dry matter production and root characters, separate sets of plants were selected at random from outside the net plot area. The length and breadth of the third trifoliate leaf from the top of the plant measured and the leaf area index was calculated using the formula given by Puttaswamy et al. (1976):

$$\text{Leaf area index} = \frac{L \times W \times 0.7320 \times NoL \times NoP}{\text{Unit land area (cm}^2\text{)}}$$

where: L = length (cm); W = width (cm); NoL = No. of leaves per plant; NoP = No. of plants per unit land area.

The soybean seed yield from the net plot area was recorded at 12% moisture and computed to kg ha^{-1}.

The experimental data for the summer and south west monsoon seasons of the two years (1996 and 1997) were pooled and statistically analysed following the procedure suggested by Gomez and Gomez (1984).

Results and discussion

Plant height

There was a progressive increase in plant height with an increase in the soil moisture supply (Table 1), which could be attributed to the effective absorption and utilization of nutrients, resulting in quick growth (Ravi Barathi, 1994). The effect of stress was more visible when irrigation was only applied at 0.50 IW/CPE, so that the plots received less irrigation water (330 and 210 mm during the summer and south west monsoon seasons, respectively). The short-term moisture stress reduced the internode elongation, and the decreased plant height could not be compensated for even at later stages (Huck et al., 1983).

The application of composted coir pith (CCP) at the rate of 12.5 t ha^{-1} created a conducive environment for the production of taller plants due to the better availability of moisture throughout crop growth and the higher availability of N and K (Bharathi et al., 1986). A nutrient mixture spray combined with either the basal or split application of N, P and K encouraged higher nutrient availability and greater absorption through increased root growth throughout the crop growth period, resulting in greater plant height.

Table 1
Plant height (cm) at 60 DAS

Treatment	I_1	I_2	I_3	C_1	C_2	Mean
F_1	50.5	58.5	61.8	55.7	58.1	56.9
F_2	53.9	62.2	65.0	59.1	61.4	60.2
F_3	55.7	64.1	67.0	61.1	63.4	62.2
F_4	57.8	66.8	69.6	63.6	66.0	64.8
C_1	53.1	61.7	64.8	–	–	59.8
C_2	55.9	64.0	66.9	–	–	62.2
Mean	54.4	62.9	65.8	–	–	–

	I	C	I × C	F	I at F	F at I	C at F	F at C
CD (P=0.05)	1.4	1.1	1.9	2.7	4.2	4.6	NS	3.8

The interaction effect clearly revealed that the availability of the moisture required for normal metabolic processes through increased relative leaf water content and soil moisture supply increased the plant height significantly (I_3C_2). Both water use factors and CCP levels had very little influence on nutrient availability and utilization, giving no visible variations in plant height due to fertilizer management practices except in F_1, where the plant height was distinctly shorter, since N, P and K were applied as basal and were exhausted even in the early stages.

Leaf area index (LAI)

The LAI determines the total assimilating area available to the soybean plant and the quantum of source that would ultimately be available for translocation to the sink. The effects of moisture stress on cell expansion and division have been known for a long time (Boyer, 1970) and the direct consequence of this effect is known to inhibit leaf expansion (Slatyer, 1978). This could be seen in the 0.50 IW/CPE treatment, where the lower irrigation frequency resulted in lower LAI (Table 2) due to reduced leaf production rate and size of leaves (Sivakumar and Shaw, 1978) and accelerated leaf senescence as an indirect effect (Fischer and Hagan, 1965). The reduction in leaf area due to the reduction in water supply was found to be permanent: leaves which were formed small remained small. Thus the effect of stress even of short duration persisted for a long period (Begg and Turner, 1976).

Contrarily, the increased LAI recorded when irrigation was carried out at smaller CPE values was obviously due to the favourable plant water relationship, inducing leaf initiation and leaf expansion (Huck et al., 1986). The incorporation of CCP increased the LAI considerably and this was closely connected with higher soil moisture content (SMC), which induced better growth and development (Scott and Batchelor, 1979) and higher nutrient status, especially due to the availability of K, as corroborated by the findings of Ravi Barathi (1994). The nutrient mixture spray, coupled either with the split or basal application of N, P and K, enhanced the leaf number and size of leaf and thereby the LAI.

Table 2
Leaf area index at 60 DAS

Treatment	I_1	I_2	I_3	C_1	C_2	Mean
F_1	3.21	3.90	4.90	3.66	3.80	3.73
F_2	3.27	3.98	4.17	3.73	3.88	3.80
F_3	3.34	4.05	4.24	3.81	3.94	3.87
F_4	3.40	4.13	4.31	3.87	4.02	3.94
C_1	3.22	3.95	4.13	–	–	3.77
C_2	3.38	4.08	4.27	–	–	3.91
Mean	3.30	4.01	4.20	–	–	–

	I	C	I × C	F	I at F	F at I	C at F	F at C
CD (P=0.05)	0.05	0.05	0.07	0.13	0.19	0.09	0.06	0.18

The availability of adequate moisture for better growth, increased leaf production and leaf expansion, due to the interaction effect of more frequent irrigation and CCP application, significantly increased the LAI. The interactive effect of fertilizer either with water use factors or CCP levels did very little to create changes in LAI.

Root length

Root studies, especially on depth distribution, aid in understanding water use, nutrient uptake and the placement of fertilizer. Soil moisture affects the root growth in most crops (Hall et al., 1990). A perusal of data on root growth reveals that when the soil water content decreased, the rate of root elongation was faster, as in the case of 0.50 IW/CPE (Table 3). Thus, the roots maintain growth despite the decrease in soil water potential due to osmotic adjustment, allowing water uptake in a drying soil (Hsiao and Acevedo, 1974). Moreover, the water uptake was proportional to the water potential difference between the root xylem and the bulk soil and to the hydraulic conductivity of the combined soil root pathway (Taylor and Klepper, 1975).

Due to CCP application, the higher SMC available in the upper layers of soil restricted the root growth in that root zone resulting in shorter root length. Either the split application of N, P and K alone or a combination of nutrient mixture spray with the basal or split application of N, P and K favoured higher nutrient availability and utilization by the soybean crop and induced greater root growth.

The interaction effects clearly indicate that the synergistic effects were more pronounced between irrigation regimes and CCP levels than between fertilizer management practices and irrigation regimes or CCP levels.

Table 3
Root length (cm) at 60 DAS

Treatment	I_1	I_2	I_3	C_1	C_2	Mean
F_1	39.7	36.8	32.0	36.4	34.3	35.4
F_2	37.2	32.9	31.5	34.9	32.6	33.8
F_3	38.0	33.5	32.1	35.7	33.3	34.5
F_4	38.5	34.1	32.7	36.3	33.8	35.0
C_1	38.6	33.9	32.9	–	–	35.1
C_2	36.1	31.8	30.6	–	–	32.8
Mean	38.3	34.3	32.1	–	–	–

	I	C	I × C	F	I at F	F at I	C at F	F at C
CD (P=0.05)	0.85	0.70	1.20	1.55	2.45	2.60	2.05	2.10

Dry matter production (DMP)

The rate of DMP is an important factor in determining the yield potential of any genotype. The increased DMP obtained from a frequently irrigated crop (0.90 IW/CPE) (Table 4) was attributed to higher SMC, which promoted favourable growth, as could be seen from the increased level of growth components (plant height, leaf area). The increased DMP due to the addition of CCP was attributed to favourable soil physical conditions, SMC and nutrient availability (Liyanage, 1989). The split application of N, P and K or the combination of nutrient mixture with either the basal or split application of N, P and K increased the DMP by providing adequate nutrients at the required time, and this phenomenon was more pronounced during the summer.

The interaction between irrigation schedules and CCP levels or fertilizer management practices, and between CCP levels and fertilizer management practices clearly showed their positive interactive influence on DMP.

Soybean seed yield

Significantly higher yield (1587 kg ha^{-1}) was recorded at 0.90 IW/CPE (Table 5). The yield increase was steep from the lowest moisture regime of IW/CPE 0.50 (1292 kg ha^{-1}) to 0.70 (1492 kg ha^{-1}). Increased soil water deficit resulted in slower growth, shorter plants (Huck et al., 1983), fewer branches with lower LAI (Fischer and Hagan, 1965), reduced dry matter production (Eck et al., 1987) and a significant reduction in the rate of photosynthesis due to the reduced leaf production rate and size of leaves (Sivakumar and Shaw, 1978), leading to the lower accumulation and translocation of photosynthates and subsequent ovule abortion, which reduced the number of pods per plant (Muchow, 1985) and ultimately the seed yield.

The effect of CCP application was more pronounced under insufficient moisture availability conditions. Being an organic material, CCP increased the buoyancy of the soil and improved the soil structure, thus providing an optimum soil environment. Moreover, the higher water-holding capacity of CCP supplied moisture in a sustained manner and alleviated moisture stress conditions, coupled with the addition of plant nutrients to the soil. These facts cumulatively increased the growth and yield attributes, resulting in a higher soybean seed yield (Ravi Barathi, 1994).

Table 4

Dry matter production (kg ha^{-1}) at 60 DAS

Treatment	I_1	I_2	I_3	C_1	C_2	Mean
F_1	2273	2491	2581	2422	2474	2448
F_2	2337	2564	2637	2487	2537	2512
F_3	2351	2581	2676	2501	2569	2535
F_4	2407	2639	2736	2554	2634	2594
C_1	2314	2532	2628	–	–	2491
C_2	2369	2606	2687	–	–	2554
Mean	2342	2569	2657	–	–	–

	I	C	I × C	F	I at F	F at I	C at F	F at C
CD (P=0.05)	37	30	52	78	124	54	39	111

Table 5

Seed yield (kg ha^{-1}) of soybean

Treatment	I_1	I_2	I_3	C_1	C_2	Mean
F_1	1199	1344	1410	1271	1364	1317
F_2	1274	1467	1557	1377	1488	1433
F_3	1300	1501	1594	1407	1523	1465
F_4	1395	1657	1797	1543	1682	1413
C_1	1221	1435	1543	–	–	1399
C_2	1363	1549	1631	–	–	1514
Mean	1292	1492	1587	–	–	–

	I	C	I × C	F	I at F	F at I	C at F	F at C
CD (P=0.05)	36	30	52	54	89	93	72	76

Another distinct feature, as mentioned earlier, was that even at the lowest level of irrigation (I_1) there was a significant yield increase with the application of CCP compared to the non-application of CCP, indicating that a seed yield increase could be obtained by the application of CCP at times of short-term moisture stress.

The split application of N, P and K combined with a nutrient mixture spray primarily facilitated the higher availability of plant nutrients throughout the crop growth period. Nutrients, especially N, promote the synthesis of proteins, organic phosphorus compounds and carbohydrates and result in better vegetative growth. P helps in increasing the test weight and K plays an important role in water uptake and in the regulation of its loss through stomatal apertures, improving the water relations in plants and thus sustaining a higher grain yield. Micronutrients regulate various physiological (metabolic) activities and help cumulatively in increasing the seed yield of soybean. By the better manipulation of nutrient feeding in the very early stage, i.e. from germination to the active vegetative stage (0-30 DAS), earlier growth was built up without wasting nutrients. The maximum vegetative stage (30 DAS) and peak flowering stage (45 DAS) were also supported by the good nutrient supply provided through the combination of basal and foliar feedings. This led to the production of higher growth and yield characters and finally resulted in higher seed yield.

The beneficial contributions of the individual factors: higher irrigation regime (0.90 IW/CPE), application of CCP and the split application of the recommended dose of N, P and K coupled with a nutrient mixture spray, had a synergistic effect and boosted the seed yield significantly in the I_3C_2, I_3F_4 and C_2F_4 combinations.

Water use

Seasonal water use was greater during the summer than during the south west monsoon season irrespective of the water use factors (Table 6). The quantity of effective rainfall decreased progressively as the water use factor increased from 0.50 to 0.90 (63.3 to 47.1 and 123.6 to 84.3 mm for the summer and south west monsoon seasons, respectively). The utilization of annual rainfall was more effective during the summer (78.2 to 58.2%) than during the south west monsoon season (68.5 to 46.8%).

The seasonal water use increased from 393.3 to 497.1 and from 333.6 to 414.4 mm during summer and the south west monsoon season, respectively. This was due to the subsequent increase in the irrigation regime from 0.50 to 0.90 IW/CPE, which added 103.8 and 80.8 mm of water to the soil in the 0.90 IW/CPE treatment, during summer and the south west monsoon season, respectively.

Table 6
Water use studies

Irrigation levels (IW/CPE)	Irrigation water applied (mm)	Effective rainfall (mm)	Seasonal water use (mm)	Rate of water use (mm day^{-1})
Summer season				
I_1 – 0.50	330	63.3	393.3	4.37
I_2 – 0.70	390	52.8	442.8	4.92
I_3 – 0.90	450	47.1	497.1	5.52
Monsoon season				
I_1 – 0.50	210	123.6	333.6	3.71
I_2 – 0.70	270	101.3	371.3	4.13
I_3 – 0.90	330	84.3	414.4	4.60

Consumptive use (mm)

Treatment	Summer season			Monsoon season		
	C_1	C_2	Mean	C_1	C_2	Mean
I_1	292.0	323.7	307.9	238.1	255.7	246.9
I_2	323.8	352.1	338.0	257.6	272.2	264.9
I_3	347.8	371.3	359.5	272.9	283.2	278.1
Mean	321.2	349.0	335.1	256.2	270.4	263.3

Data not analysed statistically

Conclusions

The study has clearly established that irrigation at 0.90 IW/CPE with the addition of composted coir pith at 12.5 t ha^{-1}, the split application of the recommended dose of N, P and K (basal and top dressing at 30 DAS) and nutrient mixture spray (2% conc.) at 30 and 45 DAS enhanced the growth attributes and resulted in higher soybean seed yields.

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INVESTIGATION OF CORRELATIONS AND MILKING PARAMETER DISTRIBUTION ON CATTLE FARMS IN EASTERN CROATIA

P. MIJIĆ, I. KNEŽEVIĆ, M. BABAN, M. DOMAĆINOVIĆ and D. RIMAC

FACULTY OF AGRICULTURE, UNIVERSITY OF J. J. STROSMAYER, OSIJEK, CROATIA

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Recently secondary cattle selection traits have been given more attention in developed cattle breeding countries in establishing a selection index. In this way, milking traits have acquired a prominent place. This paper aimed to determine coefficients of correlation and regression between a number of milking traits, that could be helpful in establishing a selection index for breeding bulls and their dams. A further goal was to determine the distribution of milking parameters. The data of 303 Holstein Friesian and 235 Simmental cows were analysed.

The results showed that in both cow breeds correlations existed between milk yield and average milk flow (0.39 and 0.49), as well as between milk yield and milking time (0.53 and 0.35). Negative correlations were found between average milk flow and milking time (–0.49 and –0.56). For the Holstein Friesian breed, 67.0% of the cows had a total milk flow in the range of 1.61 to 3.60 kg/min, whereas in the Simmental breed 72.2% of the cows had a total milk flow of 2.40 kg/min.

The milk flow rate can be indirectly affected by selecting cows with higher milk production. The definition of an optimal milk flow rate and the determination of breeding goals for milking traits will lead to faster progress in milking trait improvement and an easier choice of quality breeding bulls and dams.

Key words: correlations, milking parameter distribution, Simmental and Holstein Friesian cows

Introduction

Milking speed is a vital functional trait in dairy cows (Dodenhoff et al., 1999; Sprengel et al., 2000), the importance of which has changed: in the days of hand milking, a slow milk flow was justified by the low power consumption, whereas in the case of machine milking a short milking time is required due to the higher power consumption. One of the advantages of machine milking is the faster milk flow (Perez-Guzman et al., 1986), which is of great significance since over 50% of the total farm working hours are spent on milking (Bahr et al., 1995). Milking speed is a complex of many traits, of which average milk flow, milk yield and milking time are the most important ones (Pogačar, 1974; Holló and Babodi, 1979).

Former investigations by Trede and Kalm (1989) showed a positive correlation ($r_p = 0.26$) between milk yield and milk flow speed, enabling indirect selection, i.e. the alteration of milk flow speed along with milking capacity changes. Higher positive values ($r_p = 0.85$) were reported by Göft et al. (1994).

Since flow speed is affected by milk quantity, coefficients of regression between these two traits may be used for milk flow speed correction based on the milk amount (Pogačar, 1974; Mijić et al., 2001). Various correlations between milking traits were reported by Dodenhoff et al. (1999) between maximum and average milk flow (r = 0.84), by Bahr et al. (1995) between milk yield and milking time (r_p = 0.12 to 0.21) and by Duda (1995) between milking time and average milk flow (r_p= –0.08 to –0.10). Milking traits should be considered as both economic (Blake and McDaniel, 1978; Aumann et al., 1994) and health factors (Brown et al., 1986; Roth et al., 1998; Huth et al., 2001). A short milking time, that achieves maximal milk flow very rapidly and maintains it as long as possible is considered to be favourable for udder health. Thus, in defining the udder health index, milk flow speed and milking time are considered to be vital traits (Boettcher et al., 1998).

The present investigations aimed to compute coefficients of correlation and regression between milking traits and demonstrate the distribution of milking parameters. Milking traits are not at present included in the selection index used in the Cattle Breeding Programme of the Republic of Croatia, but could be soon, due to their increasing significance. The results of the present investigations could help to improve the Croatian cattle selection index.

Materials and methods

The investigations were made in Eastern Croatia on two farms for Holstein Friesian cows and on three farms for Simmental cows. The experiments involved a total of 235 Simmental and 303 Holstein Friesian cows, on which 2594 measurements were made from the first to third lactation. Only healthy cows with correct morphological appearance of teats were analysed. All cows treated against mastitis, with udder oedemas, in oestrus, ill, heavily wounded or exposed to some instantaneous condition affecting the daily milk yield were omitted. Alfa-Laval milking machines adjusted prior to the measurements to the same values of underpressure (48–50 kPa), pulsation relation (1:1) and strokes number (58–60) were used. Each cow underwent at least one evening and one morning measurement, and in some cases as many as six repetitions were made.

Measurements were conducted using a milk flow gauge (Tru-Flow, Tru Test$_{TM}$) in the period 50th –180th lactation day according to the German regulations (ADR, 1987). Only data on milk yields of 5 kg or more were used for the analysis. Data were collected on MY = milk yield (kg), AMF = average milk flow (kg/min) and MT = milking time (min) (Table 1).

Milking was considered to end when the average milk flow dropped below 0.2 kg/min, when the Tru-Flow device stopped further measurement and gave a light signal indicating the end of milking.

Milk flow correction was done with a model based on the coefficient of regression between milk yield and actual milk flow (Pogačar, 1984; Jakopović and Knežević, 1993):

$$CMF = AcMF + b_{xy} (MYM - \overline{x})$$

where CMF = corrected milk flow (kg/min), AcMF = actual milk flow (kg/min), b_{xy} = determined coefficient of regression between milk yield and actual milk flow, MYM = milk yield per one milking (kg), $\overline{x}$ = approximate constant calculated on the basis of milk yield in a standard milking.

The coefficient of Pearson's correlation (PROC CORR) was used to determine correlations between the variables. Statistical data analysis was conducted using the program package SAS Version 6.03 (SAS, 1988).

Table 1

Basic statistics for traits evaluated in Holstein Friesian (n = 303) and Simmental cows (n = 235). Mean value ($\bar{x}$), standard deviation (sd) and standard error of mean (SE)

Trait*	Holstein Friesian			Simmental		
	$\bar{x}$	sd	SE	$\bar{x}$	sd	SE
MY	10.79	3.82	0.09	7.96	2.54	0.08
AMF	2.71	0.90	0.02	1.87	0.71	0.02
CMF	2.86	1.04	0.02	1.94	0.88	0.03
MT	4.31	1.98	0.04	4.59	1.59	0.05

*MY = milk yield at one milking (between 50th to 180th d) (kg), AMF = average milk flow (between 50th to 180th d) (kg/min), CMF = corrected milk flow (kg/min), MT = milking time (between 50th to 180th d) (min).

Results

One of the primary aims of dairy cattle farms is to obtain as much milk as possible. This in turns requires a longer milking time. Thus, apart from its production importance, milk yield is also an important factor in evaluating certain milking traits.

For Holstein Friesian cows highly significant differences were found between the two farms for MY (t = 3.442***), AMF (t = 15.940***) and MT (t = 30.344***). Highly significant differences were also found for Simmental cows between the first and second farm for MY, AMF and MT (t = 5.363***, 20.908*** and 11.369***), between the first and third farm for MY, AMF and MT (t = 10.176***, 11.037*** and 2.085*), and between the second and third farm for MY, AMF and MT (t = 2.253*, 7.068*** and 7.068***). From these data it can be concluded that the farms had an important influence on milking traits. The t-test values given in bold were obtained by correcting the degree of freedom, because the Levene test indicated that the variances were not homogeneous.

The coefficients of correlation and regression calculated for the milking traits of Holstein Friesian and Simmental cows are presented in Tables 2 and 3.

The results showed positive significant coefficients of correlation in Holstein Friesian cows between milk yield and average milk flow ($r = 0.39^{***}$) as well as between milk yield and milking time ($r = 0.53^{***}$), while the coefficients of regression, which are absolute indicators, were positive between the compared traits. Negative significant coefficients of correlation ($r = -0.49^{***}$) and regression ($b = -0.004$) were obtained between average milk flow and milking time (Table 2).

Positive correlation coefficients were also determined between milk yield and average milk flow ($r = 0.49^{***}$) and between milk yield and milking time ($r = 0.35^{***}$) for Simmental cows, while a negative coefficient of correlation was obtained between average milk flow and milking time ($r = -0.56^{***}$) (Table 3).

Table 2

Coefficients of correlation (r_p) and regression (b) between milking traits in Holstein Friesian cows ($P \leq 0.001$)

Trait*	r_p		b	
	AMF	MT	AMF	MT
MY	0.39^{***}	0.53^{***}	0.091	16.420
AMF		-0.49^{***}		–0.004

MY = milk yield, AMF = average milk flow, MT = milking time.

Table 3

Coefficients of correlation (r_p) and regression (b) between milking traits for Simmental cows ($P \leq 0.001$)

Trait*	r_p		b	
	AMF	MT	AMF	MT
MY	0.49^{***}	0.35^{***}	0.138	13.280
AMF		-0.56^{***}		–0.004

MY = milk yield, AMF = average milk flow, MT = milking time.

The distribution of milk yield classes varied in the cow breeds investigated (Fig. 1). The majority of measurements on Simmental cows (70.1%) were in the first two classes, ranging from 5.0 to 9.0 kg, after which a continuous reduction was observed in the further classes. The distribution in Holstein Friesian cows was quite different. The majority of cows had milk yields in the first to fourth classes (from 5.0 to 13.0 kg), representing a total of 74.8%. The number was slightly reduced towards the higher milk yield classes.

The milk flow in Simmental cows was mostly in the range of up to 2.40 kg/min, with the largest number of measurements (19.9%) in the class with a flow rate of 1.61 to 2.00 kg/min (Fig. 2). In Holstein Friesian cows the distribution increased towards the faster milk flow classes. The largest number of measurements (67.0%) showed values ranging from 1.61 to 3.60 kg/min, with the greatest number of measurements (15.5%) in the 2.01 to 2.40 kg/min class. A significant number of measurements (14.9%) showed a milk flow faster than 4.01 kg/min.

Unlike the milk yield and milk flow, milking time (Fig. 3) was fairly uniform in both investigated breeds, though almost twice as many measurements indicated a milking time of less than 3 minutes in Holstein Friesian (24.0%) than in Simmental (12.3%). The most measurements showed a milking time of 3.1 to 5 minutes (56.5% for Simmental and 51.8% for Holstein Friesian).

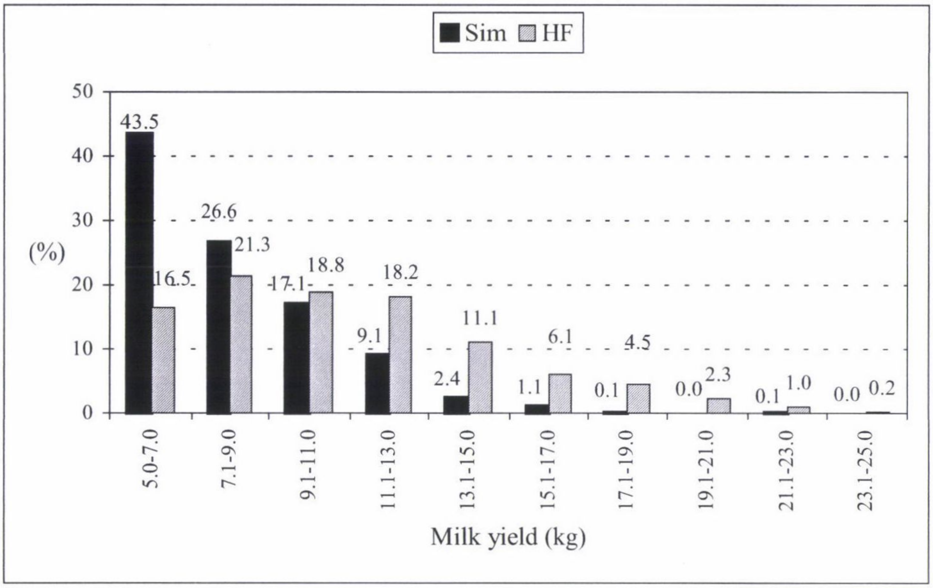

Fig. 1. Distribution of milk yield in Simmental (n=830 measurements) and Holstein Friesian cows (n=1764 measurements)

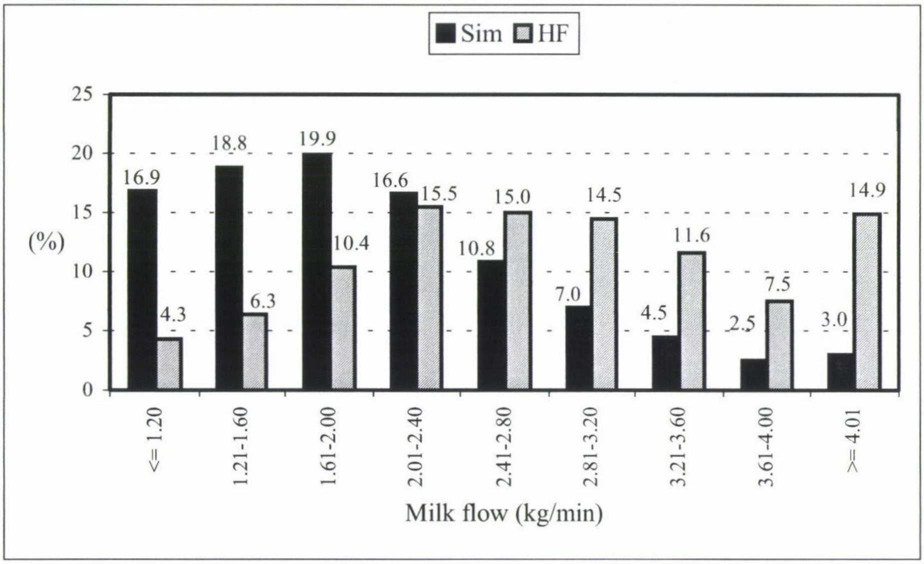

Fig. 2. Distribution of milk flow in Simmental (n= 830 measurements) and Holstein Friesian cows (n=1764 measurements)

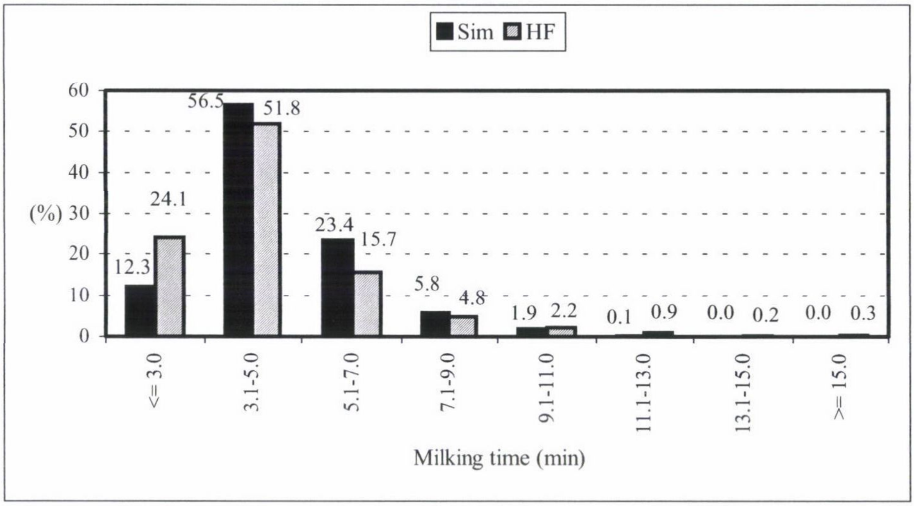

Fig. 3. Distribution of milking time in Simmental (n=830 measurements) and Holstein Friesian cows (n=1764 measurements)

Discussion

Milk flow is one of the most important parameters for the evaluation of milking speed in cattle and should be considered together with milk yield and milking time. The present investigations aimed to determine the correlation between milk flow and the other two milking parameters. The results obtained for Holstein Friesian and Simmental cows (Tables 2 and 3) showed significant positive correlations between the milk yield and average milk flow. The coefficients of correlation for these two cow breeds suggest that the milk flow speed can be affected by selecting cows with higher milk production. This fact is especially important in Simmental cows, since they were found in the present investigations to have slower milk flow compared to Holstein Friesian cows (Table 1). Optimum milk flow should thus be a breeding objective, with the determination of upper and lower milk flow speed limits as a basis for animal selection (Rügesegger, 1990; Allmen, 1994). Earlier investigations indicated a positive correlation between milk yield and milk flow, with r_p values of 0.35 (Petersen et al., 1986), 0.38 (Trede and Kalm, 1989) and 0.59 (Le Du et al., 1994). The coefficient of correlation was used for the breeding evaluation of milking speed, where a milk flow correction was done based on the milk quantity produced (Reinhardt and Dempfle, 1985; Wörle et al., 1988; Mijić et al., 2001).

The correlation between milk yield and milking time is also important in obtaining selection progress for milking speed. Coefficients of correlation between the two traits were positive in both cow breeds, which means that milking lasts longer if a higher milk yield is obtained. The same conclusion was drawn by Bahr et al. (1995), who stated that milk flow was also affected, since its speed increased together with the milk yield. The negative coefficients of correlation between average milk flow and milking time in both breeds also indicate that milking time can be reduced by selecting cows with faster milk flow.

The milk yield in Simmental cows was distributed in classes with lower values (Fig. 1), which most likely affected the milk flow speed (Fig. 2), while the milk yield and milk flow in Holstein Friesian cows were in classes with higher values. The milk flow speed distribution in Simmental cows is unsatisfactory, since 55.6% of the measurements indicated a milk flow of less than 2.00 kg/min. The percentage of measurements in the highest milk flow classes should be reduced in Holstein Friesian cows since extreme milk flow speeds may be connected with cow udder diseases (Perez-Guzman et al., 1986). These lead to a decrease in milk production and to an increase in veterinary costs, resulting in significant economic loss (Blake and McDaniel, 1978).

The positive correlations between milk yield and average milk flow mean that it is possible to influence milk flow speed through selection for increased milking capacity. Furthermore, optimum milk flow should be defined as one of the breeding goals that will be used when evaluating the milking traits of the dams of breeding bulls. On the basis of these facts it would be possible to revise breeding plan and obtain faster selection progress for improved milking traits. The coefficients of regression determined between the milk yield and average milk flow can be used for milk flow correction.

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Short communication

COMPARATIVE STUDIES ON THE SEEDLING COPPER TOLERANCE OF VARIOUS HEXAPLOID WHEAT VARIETIES AND OF SPELT IN SOIL WITH A HIGH COPPER CONTENT AND IN HYDROPONIC CULTURE

A. F. BÁLINT, G. KOVÁCS and J. SUTKA

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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On areas used for agriculture copper toxicity is one of the most important forms of heavy metal pollution, especially where field crops are to be grown in fields previously used as orchards or vineyards, treated for a long period with pesticides containing copper. Only varieties with good tolerance of soil with a high copper content should be grown on such areas. The selection of copper-tolerant varieties is complicated, however, by the fact that it is difficult to study copper tolerance under field conditions. Heavy metal tolerance is generally tested in hydroponic cultures, in which interfering factors can be minimised, but it is impossible to test a large number of genotypes or segregating generations using this method. Another problem in such experiments is that the conditions existing in hydroponic cultures bear little resemblance to those found in the field, so little information is obtained on the real adaptation of the varieties. The aim of the present experiments was thus to elaborate a soil-based technique suitable for determining the copper tolerance of various genotypes and allowing the simultaneous testing of a large number of genotypes under conditions approaching those found in the field. The results indicate that the copper tolerance of seedlings can be determined by growing them to an age of 2 weeks in soil containing 1000–1500 mg/kg $CuSO_4 \times 5\ H_2O$, since genetic differences in copper tolerance could be clearly distinguished under these conditions. The copper tolerance of plants grown in copper-containing soil exhibited a close correlation with the results obtained in physiological tests in hydroponic culture.

Key words: wheat, copper tolerance, copper-treated soil, hydroponic culture, growth chamber

Introduction

From the point of view of agriculture the most frequent and most critical forms of heavy metal pollution are those involving Al, B, Mn and Cu toxicity (Foy et al., 1978). If copper-polluted soils are to be utilised economically, without substantial yield losses, copper-tolerant wheat varieties must be grown. Previous experiments carried out in hydroponic culture proved that considerable variability existed in the copper tolerance of various wheat varieties (Tari et al., 2002; Bálint et al., 2002). The disadvantage of this method, however, is that it is very difficult to identify and select large numbers of tolerant genotypes from segregating populations in hydroponic culture and to raise a sufficient number of progeny. The logical answer would appear to be selection under field conditions on copper-polluted soil, but unfortunately this is also complicated, since polluted areas are generally located at a considerable distance from breeding institutes and the results achieved are not necessarily reliable. The results of field tests may be influenced by the occurrence of other stress factors (heat, drought, mineral or frost stress, or pathogens), so it is often difficult to clearly identify the toxic effect of copper. One way of solving this problem could be to carry out tests on artificially copper-treated soil under controlled conditions in a growth chamber.

In order to elaborate a satisfactory testing system, the present experiments were aimed at determining the best way to add copper to the soil and the best copper concentration for selection purposes. The reliability of the results obtained in soil was also checked by determining how they compared with the results obtained in the hydroponic cultures generally used to measure heavy metal tolerance.

Materials and methods

The copper tolerance of five wheat varieties (Chinese Spring, Hope, Cheyenne, Cappelle Desprez, Bánkúti 1201) and a gene bank accession of spelt (*Triticum aestivum* ssp. *spelta*) was examined in the experiments. Seeds of these varieties were soaked overnight at 20°C, then germinated for 2 days. Only seeds which were healthy and germinated at the same time were included in the experiment. The germinated seeds were sown in boxes, with 15 rows per box in a random design, as described by Veisz (1997) in the case of frost resistance testing in the Martonvásár phytotron.

The copper treatment was carried out by mixing copper sulphate ($CuSO_4 \times 5\ H_2O$) to dry soil or by spraying the soil with copper sulphate solution. The final copper concentrations were 0 (control), 25, 125, 375, 1000 and 1500 mg/kg copper sulphate in the first case, and 1000 mg/kg for the spray. In all cases the copper concentrations refer to air-dry soil quantities. The solid copper sulphate was ground to a fine powder and then sprinkled evenly on the surface of a boxful of soil (approx. 8 kg air-dry soil) spread out in a thin layer. The soil was then mixed for at least 10 min before being packed into the box. When copper was added to the soil in the form of a solution, a 10^{-4} M $CuSO_4 \times 5\ H_2O$ solution was sprayed evenly on the soil surface, adding only as much at one time as could be quickly absorbed, to avoid the solution flowing together and causing local accumulations of copper.

The seedlings planted in the soil were grown in plant growth chambers with a 16-h photoperiod, day/night temperatures of 22/20°C and 70/75 % relative humidity. The illumination was provided by Gro-Lux Cool White fluorescent tubes with a light intensity of 220 $\mu mol\ m^{-2}\ s^{-1}$. To ensure homogeneity, the boxes were moved to new positions in the chamber at random each day. The experiments were repeated four times under identical conditions.

The hydroponic experiments were set up using the method reported by Bálint et al. (2002) and the growth conditions were the same as those described for the soil experiment.

The following data were recorded in the course of the experiments: shoot length, fresh and dry mass on the 14th day after germination. The copper tolerance index was calculated as the quotient of the dry masses of plants grown under treated and control conditions (Bálint et al., 2002).

All the experiments were carried out in 3 replications with 5–25 plants per replication. The experimental data were evaluated using the MANOVA method and correlations were calculated between the results obtained in the different experimental systems. The calculations were carried out using the SPSS 8.0 program.

Results and discussion

The toxic effect of mixing copper sulphate with the soil in various concentrations was first observed at 1000 mg/kg. The lower concentrations (25, 125, 375 mg/kg) caused no visible phenotypical symptoms during the first 14 days after germination. The 1000 and 1500 mg/kg concentrations led to a significant reduction in the shoot length, fresh and dry mass of the 2-week-old seedlings compared with the control. Repeated experiments produced the same results, except that in one case the 1000 mg/kg copper sulphate treatment did not cause any significant difference between the sensitive and tolerant varieties, leading to the conclusion that the success of the experiment may be substantially modified by the physico-chemical properties and water supplies of the soil, presumably by influencing the quantity of copper available to the plants. The results suggest that a copper sulphate concentration of 1500 mg/kg is the most suitable for serial analysis.

From a technical point of view it is important to note that, according to the statistical analysis of the random design, the efficiency of the copper treatment did not depend on where the plants were situated within the box, since the responses of plants of the same genotype planted in different positions did not differ significantly from each other in any of the experiments. This means that neither the uneven distribution of the copper sulphate nor the border effect (at the edges of the boxes) caused any experimental error.

Surprisingly enough, spraying the soil with copper sulphate to a final copper concentration of 1000 mg/kg soil caused no phenotypic change whatsoever. This would suggest that the copper sprayed onto the surface of the soil accumulated in the upper soil layers and did not reach the active root zone, so that a concentration toxic to the plants was not reached in the root zone. This method is thus unsuitable for serial analysis.

The varieties tested can be divided into two groups on the basis of copper tolerance. Judging by the tolerance index, Chinese Spring, Hope, Cheyenne and *Triticum aestivum* ssp. *spelta* can be regarded as relatively copper tolerant, while two varieties, Cappelle Desprez and Bánkúti 1201, are relatively sensitive to copper (Fig. 1). As is clear from the figure, the results obtained in copper-treated soil were in very good agreement with those recorded under hydroponic conditions. The copper tolerance indexes calculated in soil were slightly higher than those found in hydroponics except in the case of *Triticum aestivum* ssp. *spelta*, which exhibited greater tolerance in hydroponics. These differences, however, were not significant in any case.

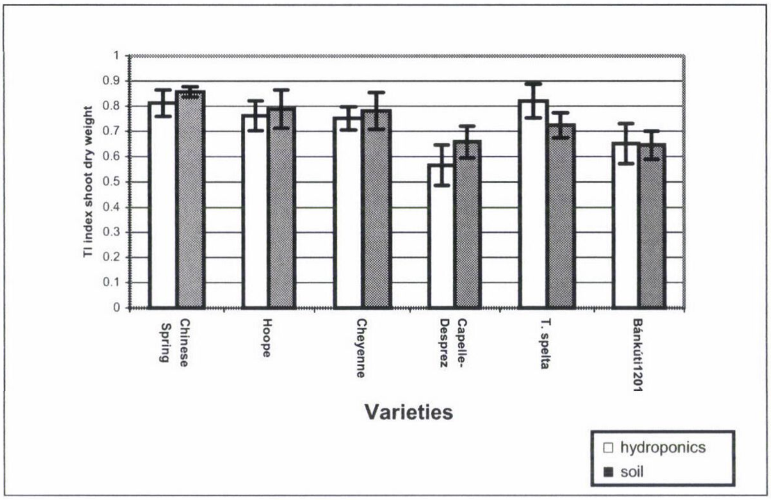

Fig. 1. Comparison of the copper tolerance of *T. aestivum* ssp. *aestivum* wheat cultivars and *T. aestivum* ssp. *spelta* in hydroponics (control: 10^{-7} M, treated: 10^{-4} M copper sulphate in the nutrient solution) and in copper-treated soil (control: 0 mg/kg, treated: 1000 mg/kg copper sulphate added to the soil)

The correlation coefficients between the two test systems indicated the presence of a close significant correlation for all the traits studied. The correlation coefficients (r) were 0.8350 (significant at the $p=0.05$ level) for shoot length, 0.9521 (significant at the $p=0.01$ level) for shoot fresh mass, 0.9069 (significant at the $p=0.01$ level) for shoot dry mass, and 0.9238 (significant at the $\alpha=0.01$ level) for the tolerance index. This proves quite clearly that the copper tolerance of wheat varieties can be determined not only in hydroponics, but also by mixing copper sulphate to the soil at an adequate concentration. The greater simplicity of this method will facilitate the selection of copper-tolerant genotypes even in the case of a large number of samples, while the chosen plants can be left to grow to maturity in the boxes. The method will also allow the toxic effects of copper to be studied in later stages of development. The results of these preliminary experiments will open the way for the genetic analysis of copper tolerance and the mapping of genes responsible for tolerance.

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Review

PHYSIOLOGICAL RESPONSES OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) TO DROUGHT STRESS AND ITS AMELIORATION: A REVIEW

T. Y. REDDY[1], V. R. REDDY[2] and V. ANBUMOZHI[3]

[1]AGRICULTURAL RESEARCH STATION, KAMALNAGAR, ANANTAPUR, ANDHRA PRADESH, INDIA
[2]USDA:ARS, BELTSVILLE AGRICULTURAL RESEARCH CENTER, MARYLAND, USA
[3]SCHOOL OF FRONTIER SCIENCES, UNIVERSITY OF TOKYO, TOKYO, JAPAN

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Groundnut (*Arachis hypogaea* L.) is an important cash crop for tropical farmers. It is an annual legume and its seeds contain high amounts of edible oil (43–55%) and protein (25–28%). Even though it is fairly drought-tolerant, production fluctuates considerably as a result of rainfall variability. To develop a water stress response function in groundnut, research has been done to improve the performance under varying degrees of stress at various physiological stages of crop growth. This review summarizes recent information on the drought resistance characteristics of groundnut with a view to developing appropriate genetic enhancement strategies for water-limited environments. It is suggested that there are considerable gains to be made in increasing yield and stabilizing the yield in environments characterized by terminal drought stress and further exploiting drought escape strategy, by shortening crop duration. Many traits conferring dehydration avoidance and dehydration tolerance are available, but integrated traits, expressed at a high level of organization, are likely to be more useful in crop improvement programs. Possible genetic improvement strategies are outlined, ranging from empirical selection for yield in drought environments to a physiological-genetic approach. It is also suggested that in view of recent advances in understanding drought resistance mechanisms, the latter strategy is becoming more feasible. It is concluded that the use of this recently derived knowledge in a systematic manner could lead to significant gains in yield and yield stability in the world's groundnut production. Research is needed to develop transferable technologies to help farmers in arid and semi-arid regions. Increasing soil moisture storage by soil profile management and nutrient management for quick recovery from drought are some of the areas which need to be explored.

Key words: groundnut (*Arachis hypogaea* L.), drought-resistant varieties, drought stress, drought-stress management, environments, water use efficiency

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop as its seed contains 44–56% oil and 22–30% protein on a dry seed basis (Savage and Keenam, 1994). Groundnut is grown on 19.3 million ha of land in 82 countries or more. More than half of the production area is in arid and semi-arid regions. Groundnut is frequently subjected to drought stress of various duration and intensity. The groundnut acreage fell by 25.8% in South Africa and 18.5% in East Africa in the 1980s compared with the 1970s. One of the reasons for the reduction in area and productivity in these areas was drought (Mahmoud et al., 1992; Fletcher et al., 1992). In India, groundnut yields fluctuated from 550 to 1100 kg ha^{-1} in different years and consequently the total production of the country also varied from 4.3 to 9.6 million tons (Patel, 1988). The rise and fall in yield and production coincided with the percentage deviation from the mean annual rainfall (DES, 1990). Early-maturing and disease- and drought-tolerant cultivars have great promise in improving production in semi-arid regions of tropical Africa and Asia. Although numerous studies have been conducted on groundnut tolerance to drought stress, no critical reviews have been written since 1990 on the effects of drought stress tolerance in groundnut and its amelioration. The existing literature on drought stress and its amelioration was reviewed to assess the present position, to identify gaps in research and to suggest future research needs. This review provides an overview of present understanding of the drought response of groundnut and summarizes current research on the enhancement of the growth and yield ability currently unrecognized in water-limited environments. In the process, strategies used previously to achieve progress in drought environments are analysed, improvements are proposed, and attempts have been made to assess the potential impacts of current research endeavours.

Drought and rainfall pattern in groundnut growing areas

India ranks first in annual total production (5.5 to 8.0 million tons) and area (7 to 8 million hectares) planted to groundnut. Other important countries in the order of total production are China, USA, Indonesia, Senegal, Nigeria, Myanmar, Sudan and Argentina (FAO, 2000). However, while the area and production of groundnut have increased throughout the world, the total productivity has remained almost constant (Patel and Golakiya, 1988). This is because rainfall plays an important role in groundnut production in many countries (Boote and Ketring, 1990). Low rainfall and prolonged dry spells during the crop growth period are the main reasons for low average yields in India. Zeyong (1992) reported that drought is the most important constraint to groundnut production in China, especially in parts of the northern region where rainfall is less than 500 mm yr^{-1}. The average yield in Australia was approximately 1250 kg ha^{-1}; however, there are reports of yields exceeding 6000 kg ha^{-1} (Middleton, 1980). Naing (1980) reported that rainfall was the main factor determining yield in Myanmar. The crop is grown in Sudan on low fertility sandy soils, mostly under low and erratic rainfall with frequent droughts. Other African countries also have considerable areas under rainfed conditions and the crop is subjected to periodic drought. The groundnut growing regions of Argentina are in a semi-arid zone and there is also great variability in the time, amount and distribution of rainfall (Pietrarelli, 1980). Hamat and Noor (1980) reported that rainfall was sufficient for the growth of groundnut in Malaysia, which has an equatorial-type climate, characterized by humidity above 60%, abundant rainfall (2000–3000 mm yr^{-1}), and temperatures ranging from 22°C to 31°C throughout the year. In Thailand, groundnut is grown in both the dry and wet seasons and the monsoon or wet season begins in May and ends in October. This is the critical time for Thai farmers, since about 80% of the total cultivated land depends mainly on rainfall (Lapang et al., 1980).

Effect of drought stress on plant growth and yield

Drought stress has an adverse influence on the water relations (Babu and Rao, 1983), photosynthesis (Bhagsari et al., 1976), mineral nutrition, metabolism, growth and yield of groundnut (Suther and Patel, 1992). In addition, drought conditions influence the growth of weeds, agronomic management, and the nature and intensity of insects, pests and diseases (Wightman and Wightman, 1994; Wheatley et al., 1989).

Water relations

Relative water content (RWC), leaf water potential (Ψ_L), stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important parameters that influence water relations in groundnut. The RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and the leaf matures (Jain et al., 1997). Obviously, stressed plants have lower RWC than non-stressed plants. The RWC of non-stressed plants ranges from 85 to 90%, while in drought-stressed plants it may be as low as 30% (Babu and Rao, 1983). The Ψ_L of groundnut leaves shows large diurnal variation, with high values in the morning when solar radiation and vapour pressure deficits are low, followed by low values around midday and a gradual rise in the afternoon (Erickson and Ketring, 1985). Osmotic potential follows the same pattern but ranges less widely than leaf water potential. The leaf and canopy temperatures of irrigated plants are generally equal to or less than ambient air temperature, but rainfed plants often have a higher canopy temperature than ambient air temperature. The transpiration rate generally correlates to the incident solar radiation under sufficient water availability. However, drought-stressed plants transpire less than unstressed plants. The same pattern was observed for stomatal conductance (Mohandas et al., 1989).

Black et al. (1985) recorded lower leaf water potential, turgor potential and stomatal conductance when moisture stress was imposed; however, stomatal conductance was more strongly affected than leaf water status. Stomatal conductance was poorly correlated with leaf water potential and soil water potential. The conservative influence of decreased stomatal conductance in unirrigated plants was negated by increases in leaf-to-air vapour pressure differences caused by associated higher leaf temperatures (Craufuard et al., 2000). Transpiration rates were therefore similar in both treatments and the lower total water use of the unirrigated stand resulted entirely from its smaller leaf area index. The Ψ_L of frequently irrigated groundnut is less than –1.2 to –1.3 Mpa, while stressed plants have leaf water potentials of –3.0 to –5.0 MPa (Bennett et al., 1984; Boote and Ketring, 1990).

Subramaniam and Maheswari (1990) reported that leaf water potential, transpiration rate and photosynthetic rate decreased progressively with increasing duration of water stress, indicating that plants under mild stress were postponing tissue dehydration. Stomatal conductance decreased almost steadily during the stress period, indicating that stomatal conductance was more sensitive than transpiration during the initial stress period. Stirling et al. (1989) found that the leaves exhibited marked diurnal variation in leaf turgor, while the pegs showed less variation and maintained much higher turgor levels, largely because of their lower solute potentials. Marked osmotic adjustment occurred in growing leaves but not in mature ones, allowing them to maintain higher turgor during periods of severe stress. This adjustment was rapidly lost when stress was released (Ali Ahamed and Basha, 1998). Bhagsari et al. (1976) reported that the water potential of leaves and immature fruits were similar under drought stress conditions. It is a general observation that under severe moisture stress conditions, young pods lose their turgor and shrivel.

Azam Ali (1984) reported that the stomatal resistance of older leaves was greater than that of younger leaves. The average stomatal resistance was 2.4 s cm^{-1} during pod development and increased to more than 10 s cm^{-1} when moisture stress was imposed. The boundary layer resistance was between 0.26 to 0.48 s cm^{-1} with a mean of 0.34 s cm^{-1}. Transpiration per unit leaf area was influenced more by stomatal resistance and the vapour pressure difference between the leaf and the air than by boundary layer resistance. The leaf area index affected the transpiration per unit land area more than any other factor. Babu and Rao (1983) examined drought stress effects on groundnut over 35 days from 20 to 55 days after sowing. Under adequate water availability, the leaf water potential varied between –0.15 MPa and –1.15 MPa at 6.00 AM and 4.00 PM, respectively. The relative water content ranged between 100% and 87% on the first day of stress imposition. At the end of this 35-day dry period the plants were wilted and leaf water potential was –5.0 MPa. The lowest relative water content recorded was 29.7%. The leaf water potential and relative water content were negatively correlated with a correlation coefficient of –0.95. The linear regression equation was $\Psi_L = 64.8 - 0.61$ RWC. Babu and Rao (1983) also stated that groundnut has the ability to recover from prolonged desiccation (to a level of about –5.0 MPa leaf water potential), indicative of moisture stress endurance. They inferred that the threshold for stomatal closure due to moisture stress in groundnut was a leaf water potential of about –1.35 MPa. Stomata are present on both sides of the leaf (amphistomatous). The upper surface has a mean stomatal number of 243 mm^{-2} in cultivar J-11 (Babu and Rao, 1983). Collino et al. (2001a, b) also confirmed that the stomatal frequency was less on the lower epidermis.

Photosynthesis

Canopy photosynthesis is reduced by moisture stress due to reduced stomatal conductance and a reduction in leaf area. As moisture stress increases, the stomata start closing as a mechanism to reduce transpiration. As a consequence, the entry of carbon dioxide is also reduced. The decrease in the conductance of mesophyll cells due to moisture stress results in low conductance of carbon dioxide and a reduction in photosynthesis. Bhagsari et al. (1976) observed large reductions in photosynthesis and stomatal conductance as the relative water content of groundnut leaves decreased from 80 to 75%. The main effect of soil water deficit on the leaf carbon exchange rate is exerted through stomatal closure. These authors reported reduced carbon exchange rate, decreased transpiration and increased stomatal resistance within three days of withholding water in potted plants. Under field conditions, Allen et al. (1976) found reduced stomatal resistance after seven days of stress and significant differences within ten days between stressed and non-stressed plants. The long-term effect of soil water deficit on canopy assimilation is a reduction in leaf area. Leaf expansion is more sensitive to soil water deficit than stomatal closure (Black et al., 1985). Drought reduces the leaf area by showing leaf expansion and reducing the supply of carbohydrates.

Reddy and Rao (1968) reported that severe drought stress decreased the levels of chlorophyll a, b and total chlorophyll. The decrease in chlorophyll was attributed to the inhibition of chlorophyll synthesis as well as to the accelerated turnover of the chlorophyll already present. However, mild drought stress increased the chlorophyll content (Moreshat et al., 1996). Stirling et al. (1989) reported that the leaves were the primary sites of ^{14}C fixation, followed by stems and pegs. The fixation of carbon was low during drought stress but increased sharply when drought stress was relieved. Under drought stress, the stems were initially the major sinks for carbon dioxide but their sink activity disappeared almost completely when stress was ameliorated. Dry matter accumulation was reduced by prolonged water deficit (Rao et al., 1985; Sivakumar and Sharma, 1986).

Carbon dioxide

The effect of elevated atmospheric CO_2, alone or in combination with drought stress, on stomatal frequency in groundnut was investigated by Clifford et al. (1995). CO_2 only exerted significant effects on stomatal frequency in irrigated plants. In droughted plants stomatal frequency was reduced by eight percent on the adaxial leaf surface only (Azam Ali, 1995). It was suggested that the effects of future increases in atmospheric CO_2 concentration on stomatal frequency in groundnut are likely to be small, especially under conditions of water stress. However, the combination of a reduction in leaf conductance and enhanced assimilation at elevated CO_2 will be important in semi-arid regions. It was also demonstrated by Clifford et al. (1993) that elevated CO_2 increased the maximum rate of net photosynthesis by up to 40% under well-irrigated conditions, and by up to 94% on a soil profile. Harvest index was unaffected by elevated CO_2 (Clifford et al., 1993; 1993b). The primary effects of elevated CO_2 on growth and yields were mediated by an increase in radiation use efficiency and the prolonged maintenance of higher leaf water potential during drought.

Anatomical changes

Periodic water stress leads to anatomical changes such as a decrease in the size of cells and intercellular spaces, thicker cell walls and greater development of epidermal tissue. The ratio of stomata per epidermal cell is predetermined, but the size of the cells is reduced without a reduction in this ratio. Therefore, the stomata per unit leaf area tends to increase under water stress. Leaves also become thicker under moderate drought stress (Reddy and Rao, 1968). The developing leaves of groundnut have an unusually thick layer of cells devoid of chloroplasts with a lower epidermis below the sponge parenchyma. The cells in this layer are considered to be water storage cells (Reddy and Rao, 1968). During moisture stress, the opposing leaflets of the trifoliate leaf come together and orient themselves parallel to the incident solar radiation, in an effort to reduce the solar radiation load on the leaf. These parahelionastic movements are common to other leguminous plants. During these movements, the upper photosynthetically active laminar regions of two opposing leaflets come together and the lower surface of the leaflets (with their disintegrated cellular layer beneath the lower epidermis) becomes exposed to sky and ground radiation (Chung et al., 1997). These air-filled lower surfaces with their lower conductivity and larger vapour diffusion path are expected to have higher stomatal resistance relative to the upper photosynthetically active layer of the leaflet. These lower surfaces also exhibit radiation reflectance properties (Babu and Rao, 1983).

Mineral nutrition and salinity

Nitrogen fixation by leguminous plants is reduced by moisture stress due to a reduction in the leghaemoglobin in the nodules, the specific nodule activity and the number of nodules. In addition, the dry weight of the nodules is significantly reduced in moisture-stressed plants. Moisture stress also delays nodule formation in leguminous crops (Reddi and Reddy, 1995). There is a considerable amount of evidence to show that the N, P and K uptake of groundnut is reduced by moisture stress (Kulkarni et al., 1988). N, P and K uptake and transpiration rate are highly correlated even under mild water stress conditions. Nitrogen assimilation is also affected by moisture stress due to a reduction in nitrate reductase activity. There is limited information on the effects of water stress on nodulation and nitrogen fixation in groundnut. Lenka and Mishra (1973) reported fewer (240) nodules per plant when irrigated at 75% depletion of available soil moisture compared to those irrigated at 25% depletion of available soil moisture where there were 553 nodules per plant. By contrast, Shimshi et al. (1967) reported no effects of irrigation frequency (7, 14, 21 days) on nodule number and nodule weight at the end of the season for groundnut grown on deep soil with high organic matter.

Leakage of solutes as a consequence of membrane damage is a common response of groundnut tissue to several types of stress including low or high temperatures, low soil moisture or high soil salinity. There is much evidence indicating that calcium is required to maintain membrane integrity (Boss and Mott, 1980). Chari et al. (1986) reported the favourable influence of calcium additions in crop water relations and tolerance to drought stress in groundnut. Enrichment of tissue with calcium results in the maintenance of a higher water status under moisture stress. The extent of membrane damage was reduced when the leaves were subjected to simulated stress in plants fed with higher levels of Ca^{++} than in leaves without Ca feeding. The rate of water loss from the leaves of Ca^{++}-enriched tissue was also lower.

Insufficient soil water in the pod zone can depress calcium uptake by developing pods and cause more unfilled pods, single-seeded pods and lower calcium content in the shells and seed (Skelton and Shear, 1971). Typical symptoms of calcium deficiency in seeds include hollow heart and damage to the embryo or plumule development. These symptoms are more prevalent in pods that are subjected to drought stress during the pod formation period (Wright et al., 1991).

Calcium is supplied to growing fruits of different crops passively through the transpiration stream. Groundnut pods, being underground, do not transpire significantly and therefore often do not receive sufficient calcium from xylem flow into the fruit (Skelton and Shear, 1971). Growing pods act as roots and absorb soil moisture that is then supplied to leaves.

Metabolism

Almost all metabolic processes are affected by water deficits. Severe water deficits cause decreases in enzymatic activity. Complex carbohydrates and proteins are broken down by enzymes into simpler sugars and amino acids, respectively (Pandey et al., 1984). The accumulation of soluble compounds in cells increases osmotic potential and reduces water loss from cells. Proline, an amino acid, accumulates whenever there is moisture stress. The accumulation of proline is greater in the later stages of drought stress and therefore its concentration is considered a good indicator of moisture stress (Reddi and Reddy, 1995).

Shoot growth

Water deficits reduce the number of leaves per plant and individual leaf size. Leaf longevity and leaf area duration are reduced by decreasing soil water potential. Leaf area expansion depends on leaf turgor, temperature and assimilate supply for growth, which are all affected by drought. Leaf and stem morphology are altered by water stress. Continuous water deficit results in fewer and smaller leaves, which have smaller and more compact cells and greater specific leaf weight (Chung et al., 1977). The main axis and cotyledonary branches are shorter in water-stressed groundnut plants. Soil water deficit reduces internodal length more drastically than node number.

Bell et al. (1993) studied the factors influencing dry matter partitioning in four diverse groundnut cultivars. The rates of dry matter accumulation in pods (pod addition) varied significantly with both cultivar and sowing date. Within cultivars, much of this variation could be attributed to variation in the crop growth rate (CGR) during the critical pod addition period. The proportion of current assimilate distribution to the pods depended on inherent cultivar characteristics, and also correlated well with current CGR relative to the CGR during pod addition. Assimilate distribution between vegetative and reproductive parts was not influenced by plant density or the spatial arrangement of the plants. All cultivars appeared capable of remobilizing stored assimilates to maintain near constant rates of dry matter accumulation in the pods (Pandey et al., 1984).

Root growth

Roots grow rapidly during the germination and seedling stages and within 5 or 6 days after sowing, the taproot may reach a depth of 10 to 16 cm and develop a number of lateral roots (Yarbrough, 1949). Groundnut roots grow rapidly, consuming a considerable portion of the early-produced assimilates. By 80 days after sowing more than 80% of the total root system is established in long duration varieties (150 days). Ketring and Reid (1993) found that root length density significantly increased at 10 cm depth until 80 days. At 40–45 days, the roots had penetrated to a depth of 120 cm and spread laterally at least 46 cm. The investigations of Gregory and Reddy (1982) indicated that the total root length of cultivar Robout 33-1 followed a sigmoid growth curve and peaked at 68 days after sowing.

The root growth of groundnut is influenced by soil moisture. Water stress stimulates the growth of roots into deeper soil (Lenka and Mishra, 1973; Narasimham et al., 1977). Allen et al. (1976) concluded from measured soil water extraction that, during water stress, roots at lower depths continue to grow deeper even though vegetative growth appears to stop. They further stated that groundnut roots effectively extracted soil water to depths of at least 180 cm in fine sand soil. Simmonds and Ong (1987) found that the cultivar Robut 33-1 extracted water from deeper layers more rapidly when grown at high vapour pressure deficits than when grown in more humid air. Devries et al. (1989) reported that cultivar Florunner had higher root length density in the deeper layers (60–150 cm) during drought periods. Florunner exhibited a greater capacity for deep rooting at 55 days after sowing than that of soybean or cowpea, especially when grown under drought stress. All these traits contribute to the ability of groundnut to avoid drought stress. Pandey et al. (1984) showed that peanut had greater root length density deeper in the soil than other legumes when grown under drought stress.

Sabale and Khuspe (1989) observed the highest root lengths when available soil moisture was 80 to 85% field capacity. They also reported that spraying antitranspirants did not influence either root length or root volume. Fertilizer phosphorus had a favourable influence on root volume but not on root length. Meisner (1991) used two non-destructive methods, a rhizotron and minirhizotron, to observe groundnut root growth under 30-day drought stress periods beginning 20, 50, 80 and 110 days after sowing. Root growth was reduced significantly by drought stress from 20–50 days after sowing compared with the irrigated control in the rhizotron study; however, such differences were not observed in plants grown in a minirhizotron (Meisner and Karnok, 1991). No other stress period had any influence on root growth.

Meisner and Karnok (1992) observed the root growth on the rhizotron glass every week and found that, regardless of water stress, the groundnut root system did not exhibit signs of senescence. Root colour and the fluorescence of the root system did not change throughout the season at any depth, indicating the viability of the roots. The ability of groundnut to maintain a viable root system during water stress may contribute to the crop's drought resistance (Sanders et al., 1993). Greater carbon partitioning to the root system before pod set, and a root system that maintains itself for a long period should be an advantage over plants whose roots are continually dying and regrowing during reproductive development.

Yield attributes and yield

The start of flowering is not delayed by drought stress (Boote and Ketring, 1990). The rate of flower production is reduced by drought stress during flowering but the total number of flowers per plant is not affected due to an increase in the duration of flowering (Gowda and Hegde, 1986; Janamatti et al., 1986; Meisner and Karnok, 1992). A significant burst of flowering on the alleviation of stress is a unique feature in the pattern of flowering under moisture stress, particularly when drought is imposed just prior to reproductive development (Janamatti et al., 1986). When stress is imposed 30 to 45 days after sowing the first flush of flowers produced up to 45 days did not form pegs during that period; however, flowers produced after re-watering compensated for this loss (Gowda and Hegde, 1986).

Peg elongation, which is turgor dependent, is delayed due to drought stress (Boote and Ketring, 1990). The pegs fail to penetrate effectively into air-dry soil, especially in crusted soils. Often, within 4 days of withholding water, the soil surface becomes too dry for peg penetration. Skelton and Shear (1971) reported that adequate root zone moisture could keep the pegs alive until the pegging zone moisture content was sufficient to allow penetration and the initiation of pod development. Once the pegs are in the soil, adequate moisture and darkness are needed for pod development. Adequate pod zone moisture is critical for the development of pegs into pods and adequate soil water in the root zone cannot compensate for the lack of pod zone water for the first 30 days of peg development. After 30 days of adequate pod zone moisture, the pods can continue normal growth in dry soil if the roots have adequate moisture. Wright et al. (1994) and Bennett et al. (1990) reported that pod formation was affected by a dry pod zone. However, Boote et al. (1992) reported that Florunner and Robout 33-1 produced pods in air-dry soil, although at a slower rate. Sexton et al. (1997) reported that peanut fruit growth was sensitive to surface soil (0–5 cm) conditions due to its subterranean fruiting habit. Dry pegging zone soil delayed pod and seed development. Soil water deficits in the pegging and root zone decreased the pod and seed growth rates by approximately 30% and decreased weight per seed from 563–428 mg. Peg initiation growth during drought stress demonstrated an ability to suspend development during the period of soil water deficit and to re-initiate pod development after the drought stress was relieved (Sexton et al., 1997)

Pod and kernel development are progressively inhibited by drought stress due to insufficient plant turgor and lack of assimilates. These stages can also be delayed by a lack of soil water in the pod zone (Boote and Ketring, 1990; Stirling and Black, 1991). Pod dry weights were significantly reduced by a 30-day water stress during the pod development stage (Meisner and Karnok, 1992). The number of pods per plant may be low due to increases in soil resistance caused by prolonged drought (Sharma and Sivakumar, 1991). Drought reduces pod yield primarily by decreasing the duration of the pod development phase (Stirling and Black, 1991). Water deficits during kernel or seed development reduce the pod and seed weight. The shelling percentage is reduced by moisture stress during seed development (Janamatti et al., 1986).

Prabawo et al. (1990) reported that irrigation applied before and/or after the early pod filling stages increased the pod yields of Spanish type groundnuts (100-day maturity) to 2.4 t ha^{-1} compared with 0.53 t ha^{-1} in a dryland crop. The dryland crop, which received no rainfall during the season, presumably extracted significant amounts of soil moisture at depths to and below 1.2 m. The pod yield of groundnut and the rainfall received during pod formation to maturity were positively correlated in a rainfed crop grown in the semi-arid region of Andhra Pradesh in India (Subbaiah et al., 1974). Suther and Patel (1992) found that pod yield was higher at 80% available soil water than at 20% available water. No pods were formed when the plants were grown in water-saturated soil (Bailey and Biosvert, 1991). Stirling and Black (1991) concluded that the major cause of variability in pod yield and harvest index in semi-arid tropics was the delay between peg initiation and the onset of rapid pod growth. The reason for this was that once pods were initiated, the proportion of dry matter allocated to reproductive sinks was relatively constant.

Moisture-sensitive stages

It is essential to identify the moisture-sensitive developmental stages in order to minimize damage caused by drought. The pre-flowering phase is less sensitive to moisture stress than the flowering phase. Greater synchrony of pod set in moderately stressed plants during the pre-flowering phase resulted in a higher proportion of mature pods at final harvest (Kulkarni et al., 1988; Rao et al., 1988). Yield reductions were the greatest when stress was imposed during the period between pegging and pod development, and the lowest when stress was imposed from pod development to maturation (Patel and Golakiya, 1988). Several reports indicate that the pod development phase is the period most sensitive to moisture deficit (Stirling et al., 1989; Patel and Gangavani, 1990; Meisner, 1991; Ramachandrappa et al., 1992). Irrigation timing affected pod yield mainly by influencing the duration of pod production. Naveen et al. (1992) found that water stress imposed during the flowering and pegging stages of JL-24 produced the greatest reductions in pod yield followed by water stresses at the early and late pod stages. In JL-24 the deviation is probably due to the shorter duration of flowering.

Seed viability

Nautiyal et al. (1991) subjected groundnut cultivars to soil moisture stress at different growth stages and reported that moisture stress during the early vegetative phase resulted in an increase in individual seed weight. Stress at the pod initiation and pod development stages reduced germination, vigor, seed membrane integrity and embryo RNA content. Moisture stress at pod development resulted in seeds which, on germination, had low chlorophyll and dehydrogenase activity in the cotyledons. The growth potential was linearly related to the chlorophyll content and dehydrogenase activity during seed germination.

Quality

Groundnut seed contains approximately 50% oil. Generally, the oleic and linoleic acid together make up 80% of the fatty acids in groundnut oil. Groundnut storage qualities and nutritional quality are both dependent on the relative proportion of saturated and unsaturated fatty acids in the oil. From the human nutritional stand-point, a high polyunsaturated fatty acid content is desirable for lowering the plasma cholesterol level. Fats containing a higher percentage of oleic acid are also beneficial in lowering blood cholesterol. The total amount of unsaturation is inversely proportional to the storage life of the oil. Drought stress during the maturation period results in a decreased oleic: linoleic ratio and less stable oil (Hasim et al., 1993). Sharma and Singh (1987) reported that the oil content in groundnut cultivar M 13 was not influenced by moisture stress. Conkerton et al. (1989) reported that drought stress early or late in the growing season had little or no effect on seed oil proteins and mineral contents in the eight groundnut varieties tested. Drought during mid-season affected all these components, but only the decrease in oil and copper content were consistent for all cultivars.

Pettit et al. (1971) observed that groundnut grown under dryland conditions and subjected to drought, contained more aflatoxin than groundnut grown under irrigation. Wilson and Stansell (1983) reported that water stress during the last 40 to 75 days of the season contributed to the aflatoxin contamination of mature kernels. Sanders et al. (1993) reported that aflatoxin was consistently found in groundnut when the pods were exposed to drought stress, even if the roots of these plants were well watered. Generally, aflatoxin was not found in groundnut pods when the pod zone was well watered, even when the root zone was subjected to drought stress conditions.

Pests and diseases

Drought stress has a considerable influence on the weeds, insect pests and diseases of groundnut. Drought stress during vegetative growth (up to 30 days after sowing) is considered advantageous. Early drought reduces the weed population. In India, after sowing groundnut with a seed drill, the seeds are covered by running a blade harrow to a shallow depth. This operation uproots germinating and germinated weeds as well as loosening the top 3–4 cm of soil. Weeds cannot germinate from the topsoil as it dries out quickly. If the dry period continues for 20–30 days, no weeds will germinate except those from deeper layers.

The degree of insect pest infestation is also affected by drought stress. Wheatley et al. (1989) observed three distinct patterns of the distribution of foliar feeding insects. The leaf miner *Aproaerema modicella* was found in the densest numbers on the most drought-stressed plants of groundnut where leaf surface temperatures were the highest. The cicadellid *Empoasca kerri* concentrated where there was no drought stress and leaf temperatures were lowest. Thrips were initially more abundant on the plants that were least stressed, but as the condition of the plants worsened, their distribution reversed. Biochemical changes occur in plants when they become drought stressed. These changes include increases in the levels of soluble carbohydrates and amino acids in the leaf. These drought-induced changes provide a more favourable diet for insects, especially phloem feeders. Hence the often-observed phenomenon that insects feeding on drought-stressed plants have higher reproductive and development rates. Bud necrosis disease was observed more often on drought-stressed plants, and plant mortality was high due to the disease and to insufficient moisture.

Indirect influence of drought

Drought is generally accompanied by low relative humidity, high temperature and high wind speed, which also influence groundnut. Simmonds and Ong (1987) found that the transpiration of groundnut was strongly influenced by the vapour pressure deficit, which typically ranges from 1 to 5 kPa in semi-arid regions. When the vapour pressure deficit exceeded 2 kPa, canopy evaporation was restricted. The transpiration rate per unit leaf area increased with an increase in vapour pressure deficit, implying that any restriction in transpiration through stomatal closure at high vapour pressure deficit was outweighed by the steeper vapour pressure gradient from leaf to air.

Developmental processes such as time of flowering, pegging and pod formation were unaffected by different levels of vapour pressure deficits, but the numbers of branches, flowers and pegs were reduced in the drier treatments. Measurements made during the first 30 days showed that in drier environments leaf growth was reduced, and the partitioning of dry matter into the roots was enhanced. Under unirrigated conditions, the dry matter production in the shoots was reduced by 40% compared with that of well-watered plants as the vapour pressure deficit increased from 1.0 to 3.0 kPa. Growth was reduced by reductions in the leaf area, light interception and productivity per unit of light intercepted (Ong et al., 1987; Isoda et al., 1996). Ketring (1984) reported that a day temperature of 35 °C reduced the individual leaf area and dry weight of well-watered groundnut at both 63 and 91 days after sowing. High temperature also reduced the total leaf area, number of pegs and seed weight.

Drought stress management

There are several farming practices that could insure the successful production of groundnut under arid and semi-arid conditions. The following drought management strategies are recommended as practices to overcome drought stress.

Land treatments

Groundnut yields were increased by 10–20% in an arid region of Andhra Pradesh, India with contour cultivation compared to cultivation along the slope. Dead furrows are plough furrows made between the rows of groundnut around 20–30 days after sowing at an interval of 3.6 m, after every 12 rows of groundnut sown with a row spacing of 30 cm. Dead furrows are formed only when contour cultivation is practised. These dead furrows hold the run-off water and increase the soil moisture storage. This practice is suitable for the arid tropics (Reddy et al., 1993). Deep ploughing once every 3 years with a country plough (draft plough) increased infiltration and reduced runoff, resulting in increased root proliferation and groundnut yields (Munaswamy et al., 1993).

Shelterbelts

Shelterbelts are rows of trees and shrubs planted perpendicular to the direction of the prevailing winds to reduce wind velocity, evapotranspiration and wind erosion. Reddi et al. (1981) reported that shelterbelts reduced evapotranspiration and increased the yield of groundnut by 40–43%. The influence of shelterbelts was observed both on the leeward and windward sides. Similarly a maize crop can act as a shelterbelt and increase the yield of rainfed groundnut.

Mulching

Locally available plant materials can generally be used as mulch to reduce evaporation and runoff and to increase infiltration. Mulching also reduces weeds. The application of groundnut shells at the rate of 5 t ha^{-1} 10 days after the sowing of groundnut in arid regions of Andhra Pradesh, India increased the pod yield by 15–26% and the haulm yield by 17% (Reddy, 1994; 1995). White materials like groundnut shells, sand, and white stones reflect solar radiation and may reduce evaporation from the soil (Baungardener et al., 1985).

Application of amendments

Field experiments were conducted to study the direct and cumulative effect of sand application to alfisols on the soil physical properties and the productivity of groundnut in arid tropics. These results revealed that the soil infiltration rate was increased by 78% over the control treatments by the application of sand at 30 t ha^{-1} before sowing. The crust strength of the top 2–5 cm soil layer was decreased by 18–40% in the first year of sand application (40 t ha^{-1}) compared with no sand application. A higher proportion of mature pods to total pods was observed with sand application due to the reduced soil mechanical resistance in the pod zone (top 2–5 cm of soil). The pod yield increased by 13–57% over the control after sand application. The cumulative effect of sand application was observed for up to 4 years. The harvest index was improved by 6% with sand application as compared to no sand application (Reddy, 1994; Munaswamy et al., 1995). Reddi et al. (1979) found that the application of powdered groundnut shells at 5 t ha^{-1} before sowing increased the pod yield significantly during a drought year.

Drought-resistant varieties

In general, the sensitivity of a given genotype to drought increases with increasing yield potential (Narasimham et al., 1977). Genotypic variation also exists for water use efficiency (WUE, g DM kg^{-1} water), with some cultivars being able to accumulate up to 30% more shoot dry matter than others with the same total transpiration (Williams et al., 1986; Dwivedi et al., 1996; Rucker et al., 1995). Studies conducted at ICRISAT revealed that specific leaf area (SLA) and photosynthetic activity were correlated (ICRISAT, 1992). Genotypes with low SLA had high levels of the photosynthetic enzyme ribulose 1–5 bisphosphate carboxylase per unit leaf area, suggesting the enzyme was a major cause for variation in WUE (ICRISAT, 1992). WUE and leaf thickness are correlated in groundnut genotypes. Wright and Rao (1992) considered WUE as an important trait for the selection of drought-resistant varieties. They reported a close negative relationship between SLA and carbon isotope discrimination (ratios of carbon isotopes $^{13}C/^{12}C$) in the leaves. They suggested that drought-resistant genotypes could be selected either by specific leaf area or by carbon isotope discrimination. In their study, the cultivar Tifton 8 had the highest (3.71g kg^{-1}) and Chico the lowest (1.81g kg^{-1}) WUE.

Variation in the partitioning of dry matter to the pods has been reported (Greenberg et al., 1992) and it was observed that the large variation in the response of genotypes to midseason drought was due to recovery differences after drought was relieved (Williams, 1994). Schilling and Misari (1992) reviewed work done on drought in Senegal and reported four techniques for the evaluation of genotypes for drought resistance: protoplasmic resistance to heat and drying, measurement of electrolyte escape, measurement of water loss from detached leaves, and rooting characteristics measured in a rhizotron.

Dhopate et al. (1992) reported that cultivar JL-24 was more drought resistant than cultivar TAG-24. The yield reduction due to drought for JL-24 was 32.1% of the yield obtained under adequately irrigated conditions, while the yield reduction was 46.7% for the cultivar TAG-24. Joshi et al. (1988) studied two Spanish bunch cultivars, GG-2 and JL-24, and found that GG-2 had higher RWC before, during and after stress compared with JL-24. Leaf water potential was also significantly lower in GG-2 than JL-24. After re-watering, the leaf water potential returned to close to pre-stress levels in GG-2, while JL-24 did not recover. Generally, drought-tolerant cultivars have lower (more negative) water potentials but higher RWC. The transpiration rate of GG-2 was twice that of JL-24 both during stress and after stress relief. Koti et al. (1994) found that the genotype Dh-3-30 was more tolerant to drought compared with TMV 2 and had a lower transpiration rate and maximum diffusive resistance when stress was imposed by withholding irrigation for seven days for plants grown in pots during the summer season. However, the pod yields of the two varieties did not differ significantly. Four groundnut genotypes grown in medium deep alfisol in central India transpired similar amounts of water (219–

228 mm) over the season but produced different amounts of shoot dry matter (389–493 g m^{-2}) (Mathews et al., 1988). Taproot extension rates were higher for the cultivar Kadiri-3 in the first 32 days after sowing. TMV-2 and Kadiri 3 produced higher pod dry weight compared to NCAC 17090 and EC 76446.

Virginia-type cultivars typically have greater water use efficiencies, while Spanish and Valencia cultivars are superior in partitioning dry matter to the pods (ICRISAT, 1992). Genotypes belonging to the Virginia bunch and Virginia runner types, with small dark leaves, are more drought-resistant based on wilting score, while the rate of recovery from drought is faster in genotypes belonging to the Spanish bunch type. However, Erickson and Ketring (1985) suggested that Spanish types are more tolerant to drought than Virginia types under severe stress and high evaporative demand. Drought-resistant varieties typically showed a smaller decrease in RWC per unit decrease in leaf water potential compared to susceptible cultivars. Osmotic adjustment has been suggested as a mechanism that leads to smaller changes in RWC per unit decrease in leaf water potential and consequently helps to maintain positive turgor potential during water stress. A Spanish-type cultivar, Comet, gave significantly higher yield than Florunner (Virginia-type) under rainfed conditions. They concluded that the lower leaf water potential, greater change in osmotic potential and higher pod yield of Comet was related to a greater resistance to dehydration when soil moisture deficits and high evaporative demand conditions occur.

Reddy and Setty (1995) reported that ICGS (E)-198 and K-134 could be considered as drought-resistant varieties based on wilting score and leaflet angle during drought stress. The rate of recovery after drought stress was faster for TMV2, which grew at 1.66 cm day^{-1} during the first week after stress relief, compared to K-134 (0.4 cm day^{-1}). Considering various parameters such as growth during drought, rate of recovery after drought, pod yield and haulm yield, ICGV 86699, K-134 and TMV-2 were all considered to be drought-resistant varieties suitable for arid regions. Nigam et al. (1991) reported that groundnut cultivar ICGS-1, released from ICRISAT, had moderate recovery from mid-season drought. Manoharan et al. (1989) developed VRI-2 from a cross between JL-24 and CO-2. VRI-2 is a drought-resistant variety and produces an average pod yield of 1.79 t ha^{-1} under rainfed conditions. Ali and Malik (1992) reported that ICGS (E) 52 and ICGS (E) 56 were promising short-duration varieties suitable for the rainfed areas of Pakistan and could escape end of season drought due to their short duration.

Mahmoud et al. (1992) stated that the most important advance in rainfed groundnut research in eastern Africa was the release of the US line EM 9 in 1987 as a new variety, Sodiri, to replace Barberton. In 12 trials over three seasons and four locations, the new variety out-yielded Barberton by an average of 20.5%. The new varieties released in Ethiopia were NC 4X, NC 343 and ICG 94. They gave yields ranging from 2 to 5 t ha^{-1} under rainfed conditions. Schilling and Misari (1992) reported that intensive research done in western African countries had resulted in the release of several drought-resistant, short-duration and erect varieties. The variety 55-437 was released for cultivation in the dry zones of Niger, Nigeria, Chad, Gambia and Cameroon. SAMNUT-18, which had 55-437 as one of its parents, is another variety released for the region. Other drought-resistant varieties released in West Africa are: 73-30, Te-3, Ts-32-1 and 73-73. Drought-resistant varieties suitable for Botswana are: 55-437, 73-30, Flower 11, GG 8-35, ICGS (E) 30 and ICGS (E) 60.

Supplemental irrigation

Prolonged dry spells during the pod and kernel development stages can cause irrevocable loss in pod yield and one or two supplemental irrigations during these critical stages are known to increase yield (Reddi and Reddy, 1995). A rainfed crop grown in the arid tropics of Andhra Pradesh, India was given one supplemental irrigation of 5 cm of water during a dry period lasting more than 15 days. One supplemental irrigation of 5 cm during a 25-day dry period after sowing did not influence pod and haulm yields. The same quantity of irrigation water given during the pod development stage increased the pod yield by 27% and the haulm yield by 24% over the control (Reddy, 1994; 1995). Sharma and Singh (1987) found that the number of gynophores and pods plant^{-1}, 100-seed weight, pod yield and shelling percentage were highest with two supplemental irrigations at 50 and 80 days after sowing and were lowest under rainfed conditions. Irrigation at 80 days after sowing was more effective than irrigation at 50 days. However, the oil content was not influenced by moisture stress.

Seed hardening

There are reports of the long-lasting effects of seed hardening on the germination and subsequent growth of groundnut. Arjunan and Srinivasan (1989) found that dry matter accumulation and pod yield depended on the chemicals used for seed hardening and their concentration, which varied between cultivars. In general, seed hardening with 1% calcium chloride or 2% KH_2PO_4 was most effective. However, germination was adversely affected by seed treatment with 1.5% succinic acid. Bharambe et al. (1993) found that the hydrophilic polymer *Jalasakti* did not influence pod yield, either as seed treatment or as soil application.

Plant population

Wright and Bell (1992) reported that a densely planted crop extracted water from lower depths sooner than a low-density crop. Reproductive development was strongly influenced by plant population density, with more pods m^{-2} in low than in high-density crops. Lower leaf water potential and individual leaf photosynthetic rates in the middle of the day during the pegging and early podding phases suggest that high crop water deficits lowered assimilate availability and reduced reproductive potential in high compared with low density crops (Funderburk et al., 1998). They suggested that reducing plant density improved the pod yield of groundnut grown on residual soil moisture. The irrigation timing of water rather than the total amount of water applied was a major determinant of pod yield. Wright and Bell (1992) reported that dry matter production under moisture stress conditions was maximized at 40,000 plants ha^{-1} compared to production in higher plant populations. The short duration Spanish cultivar, McCullin, showed a yield response up to 80,000 plants ha^{-1}, but the Virginia cultivar, Early Bunch only up to 40,000 plants ha^{-1}.

In a very dry season on sandy soils at Sabele, Botswana, yields of a Spanish variety were highest at a density of 166,000 plants ha^{-1} and lowest at 37,000 plants ha^{-1}. Very low density led to prolonged flowering, uneven maturity and low shelling percentage (Mayeux and Maphanyane, 1989). The optimum population for Spanish bunch cultivars under rainfed conditions in India is 333,000 plants ha^{-1} (NARP, 1992). Generally crops grown on residual moisture should be planted at lower populations than those grown during the rainy season.

Antitranspirants

Naveen et al. (1992) reported that spraying 3% kaolin during dry periods at 35 and 55 days after sowing reduced the adverse affect of drought on the groundnut crop and resulted in a 139% yield increase over controls. Spraying kaolin (5%) during drought stress in the pod development phase increased the pod yield over the control. Lime, an easily available material, gave significantly higher pod yield over the control when sprayed at 1% on moisture-stressed plants (Reddy, 1994; 1995).

Conclusions

A review of the literature on drought stress and its amelioration in groundnut revealed that sufficient information is not available on respiration, nodulation, hormonal relationships and anatomical changes during drought to come to any meaningful conclusion. The response of groundnut flowering to drought has been well studied, but the growth of pods under drought stress, and the addition, degeneration and retranslocation of carbohydrate are not well understood. Similarly, the influence of soil physical conditions on the growth of pods, especially during drought needs further exploration. One tenth of the area under groundnut is situated on shallow Affisols whose water-holding capacity is extremely low. Ways to increase water-holding capacity require further study. The reflectant type of antitranspirants reflects the entire solar radiation spectrum including photosynthetically active radiation. Materials with reflecting properties in the infrared region of solar radiation may reduce transpiration without reducing yield. The screening of antitranspirant materials and the feasibility of their application in groundnut should be examined. Detailed studies are necessary to determine the effect of drought stress on nitrogen fixation, and to find methods of supplying nutrients for the quick recovery of drought-stressed plants. Most drought amelioration measures reported are related to the use of antitranspirants, supplemental irrigation and seed hardening. Antitranspirants of the reflectant type are only useful as a method of preventing crop death under severe drought, but do not increase yield. Supplemental irrigation, if provided at the pod development stage, increases yield, but the availability of water for irrigation is often limited. Seed hardening techniques rarely increase the yield by more than 10%. Some drought amelioration measures, such as drought resistant varieties and nutrient management, appear promising. Simple techniques are needed for farmers in economically disadvantaged regions.

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Review

ROLE OF OPEN-POLLINATED POPULATIONS IN THE DEVELOPMENT OF MAIZE LINES WITH COMMERCIAL VALUE

G. HADI

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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If hybrids with better yield potential than that of currently grown hybrids are to be developed, new lines will be required with better genes and gene combinations. New character combinations only arise in populations. The probability of developing lines suitable for the development of commercial hybrids from heterozygotic populations is always extremely low. This is probably due to the fact that the linkage groups of the genotype carrying favourable properties are not fixed. The method by which linkage groups can be fixed has been known for several hundred years: continual selection aimed at stabilising and standardising the desired characters, and partial inbreeding. A great deal of breeding experience provides evidence of the fact that the linkage groups containing the desired characters are not necessarily confined to a single chromosome. The joint inheritance of several chromosomes over a number of generations suggests the presence of an as yet unknown mechanism which helps to preserve the favourable characters tested and accumulated by breeding or natural selection in a system offering a number of alternatives.

Key words: maize, population improvement, inbred line development, linkage group, evolution

Introduction

In countries where modern technologies are applied, the average maize yield is around 6–8 t/ha. Under good growing conditions, however, today's varieties are capable of attaining a yield average of 12–15 t/ha, while in cases where the available water, heat and soil fertility do not limit the yield, figures as high as 22–24 t/ha have been recorded (Duvick and Cassmann, 1999).

Both the possibilities available and the consumer demands tend to favour a further increase in yield averages, to which breeders contribute to the extent of approx. 70 kg/ha/year by developing new generations of hybrids (Duvick, 1977; 1992; Russell, 1986). If hybrids better than those currently produced are to be developed, it will be necessary to develop new lines which themselves contain the desired genes or gene combinations.

In order to promote the recombination of useful traits, breeders develop populations. Most frequently this involves a cross between two related or non-related elite lines known to combine well with the chosen heterosis source, followed by the breeding of new inbred lines from the F_2 generation. This method was named pedigree breeding by Hayes and Johnson (1939) and Johnson and Hayes (1940), but has been used in practice for a very long time, as is witnessed by the records of the breeding of Arabian thoroughbreds, which were engraved on stone many thousands of years ago.

Breeding populations are developed using various components for widely differing purposes and include synthetic varieties, variety populations and gene pools. Following the suggestions of Jenkins (1940), Hull (1945), Comstock et al. (1949) and others, these populations can be improved in a closed system. Population improvement aims to increase the frequency of genes or gene combinations responsible for desirable traits, while also increasing the frequency with which lines can be obtained from the improved populations, as sources, for the development of hybrids better than those currently available.

The large number of papers on population improvement published so far report on substantial advances in the frequency of genes with a favourable effect on grain yield and other agronomic traits. Nevertheless it is interesting to note that in the compilation made by Gerdes et al. (1994) the last elite inbred lines successfully developed from heterozygotic populations are listed as C 103 (1949), B 14 (1953), B 37 (1958), B 73 (1972) and B 84 (1978). For some unknown reason, attempts to develop elite lines from population improvement have consistently failed.

In contrast, the pioneers of hybrid maize breeding, such as B. H. Duddleston, M. T. Jenkins (cit: Troyer, 1999), Cauderon, E. Pap and M. Vukovics (cit: Németh, 1985), successfully developed a number of widely used lines with commercial value from various open-pollinated varieties, in some cases developing several from a single variety. It is true that in the 1930s there was nowhere near as great a difference between the performances of source populations and commercial hybrids as there is today. The breeders mentioned often achieved these excellent results near the start of their career. It would appear that the open-pollinated varieties they used as sources differed in some respect from those used by other breeders.

Genetic linkage

In maize the various plant characters are fixed on 10 pairs of chromosomes, i.e. in 10 pairs of "linkage groups". The exact location of many monogenic (generally recessive) characters is already known. The more complex a character, however, the more data exist indicating that the interaction of many genes, often located on all 10 chromosomes, is required if the character is to be manifested. For instance, genes sh_2 (chromosome 3), su_1 (chromosome 4), ae (chromosome 5), su_2 (chromosome 6), sh_1 and wx (chromosome 9) have all been proved to play a role in the synthesis and accumulation of starch.

Genes influencing such important agronomic traits as grain yield, adaptability and stalk strength are located on several chromosomes. The breeder works with a phenotype possessing a particular set of chromosomes carrying both desirable and undesirable traits or combinations from the point of view of selection.

Many authors consider that the linkage groups inherited from the parents should be broken up by means of random mating in order to allow new linkage groups to evolve.

Lonnquist (1974) carried out random mating on adapted × exotic sources for several generations in an attempt to exploit exotic character combinations. Slow, patient selection was applied to give recombinant traits a chance to form linkage groups. It was hoped that the smooth functioning of these recombinant trait groups would lead to the appearance of extremely high-yielding plants in the population. However, this author made no attempt to fix the novel linkage groups, and unfortunately high-yielding plants disappear from the population as quickly as they arise.

Troyer and Brown (1976), Troyer (1978) and Troyer and Larkins (1985) used a geographical approach accompanied by mild selection to adapt nearly 200 non-adapted, exotic populations with a long vegetation period to more northerly growing sites. Selection for early silking also led to improvements in numerous correlated traits and in the yield. Despite the fact that the recombinant chromosomes functioned smoothly as the result of mild selection, no lines of commercial value could be produced from these sources (Troyer, 1990; 1999). This suggests that genes for early silking and high yield had not been successfully concentrated or linked in a single genotype.

Hallauer (1978) compared the breeding value of populations containing 0, 25, 50, 75 and 100% exotic blood. The best proved to be BS 16 (adapted ETO), which contained 100% exotic blood and thus probably possessed the linkage groups characteristic of ETO intact. The breaking up of linkage groups which have developed over a long period by means of human or natural selection is not always desirable.

Lamkey et al. (1995) tested 100 S_1 lines each from the B 73 × B 84 F_2 and B 73 × B 84 F_2 Syn-8 populations. Random mating over 8 generations probably broke up the linkage groups originally characteristic of B 73 and B 84, but the advantage of this was not manifest in the S_1 hybrids in the form of either a high yield average or greater yield deviation.

Phenotypic selection for desirable gene combinations

Selection for linked gene groups can be carried out on the basis of phenotypic manifestation.

Bauman (1977) was the first to report that in the course of line development an experienced breeder could develop new lines completely similar to one of the parents without having recourse to backcrossing.

According to Hadi (1993), favourable gene combinations which accumulated over a number of line generations and which proved to result in good performance could and should be preserved during line development by selection for a phenotype similar to that of the parent. The new line resembles one of the elite parental lines both phenotypically and genotypically because the majority of its chromosomes originate from this parent. The small number of chromosomes originating from the other parent, or more importantly gene exchanges between the parental chromosomes, can be tested against the "known" genetic background in an attempt to make further improvements in the favourable gene combination.

Development of linkage groups based on favourable gene combinations

During the period when open-pollinated varieties were grown, the seed had to be renewed from time to time. Although the seed used for variety maintenance was a mixture of the grain from a large number of plants in a large population, a reduction in the grain yield could often be observed after a number of years. It seems unlikely that this rapid deterioration could have been caused by a rapid, substantial change in the gene or genotype frequency due to inbreeding and/or outcrossing. If the reasons for this rapid deterioration are investigated, in addition to errors in variety maintenance, attention should also be paid to the failure to fix the characters associated with the desired performance level.

The method used to fix desirable traits was stabilising, standardising selection, which came under attack from many quarters in the early decades of the 1900s. Stabilising selection, which involved the selection of ears uniform as regards shape, size, weight, kernel row number, kernel type, etc., was often used for the maintenance of widely sown varieties. Other types of ears were ignored when forming the seed mixture used for sowing, even if they had favourable characters (Cs. Lázár, 1899; Grabner, 1908; Zathureczky, 1910; Fleischmann, 1913a, b; Troyer, 1999). The varieties with the biggest, most uniform ears were the most popular.

However, stabilising selection for uniform ears also had disadvantages, which became the focus of attention. Stabilising selection also led to a decline in genetic variability for other major characters, thus reducing the chances of achieving improvements in yield potential (Hallauer, 1990).

Stabilising selection combined with the isolated multiplication of seeds from individual ears with outstanding yields constitutes partial inbreeding for the desired gene combinations, and if carried out consistently for several generations will lead to the fixing of the selected traits.

Prof. L. Burnett of Iowa State University developed the Iodent variety by multiplying a hybrid of ear progenies 203 and 119, originating from ear-to-row improvement of the Reid Yellow Dent variety (Troyer, 1999). Fleischmann (1913a, 1939) also used the ear-to-row method to select ear No. 122, probably from a mixed population of Livingstone's Early Golden variety found in Slovenia. Repeated multiplications of the same ear culture in 1910, 1911 and 1912 led to the development of the varieties Rumai Yellow Dent, Vukovári Yellow Dent and Béllyei Yellow Dent, followed a few years later by "F" Early Yellow Dent and "F" Mezőhegyesi Yellow Dent, all of which were grown on a wide area. It is worth noting that several breeders each produced a number of lines with commercial value from 4 of these 5 varieties. The Iodent variety was used by Jenkins (1935) to develop lines I. 159, I. 205, I. 224, I. 234, etc., which were widely used for the development of commercial hybrids. In a similar manner, when E. Pap realised in 1933, after 15 years of ear-to-row breeding on the variety Mindszentpusztai Yellow Dent, that the yield of this variety could not be improved with this method, he started developing inbred lines (cit.: Hegedüs, 1996). From the first samples four widely used lines were developed (01, 014, 0118b, 156) (Németh, 1985). Later, however, despite wide-ranging sampling from this population, neither he nor other breeders were able to develop such successful lines, since later stages of variety maintenance were not aimed at stabilising the variety, and genotypes carrying favourable characters became merged in the population.

Inheritance of linkage groups

According to Mendel's law of independent assortment, in the case of a large number of progeny, the parental chromosomes will be distributed in random combinations in the progeny.

Over 20 synthetic varieties were developed in Martonvásár between 1976 and 1990 from elite and non-elite line components for various purposes. Prior to recurrent selection, random mating (sib pollination, isolated cultivation) was carried out for several years in order to mix the genes of the various line components. It was found that plants extremely similar to the elite line components (A 632, B 37, Mo 17, OH 43, etc.) were still present in the synthetic variety in a high ratio even after 3–4 random pollinations. By contrast plants resembling the non-elite line components often disappeared from the population even after the first random pollination. This phenotypic similarity to the elite lines was often observed for a considerable number of character groups. For example, if the seedlings were found to resemble one or other parental line, the similarity increased in the appearance of the tassel, leaf and stalk in the adult plant, and was most apparent in the ear and kernels. S_1 lines started after 3–4 generations of random pollination and exhibiting similarity to one of the parental lines thus gave a sister cross effect, which could certainly not be expected on the basis of Mendel's law.

This retarded mixing of the genetic material of various elite lines even after several generations suggests that the parental characters, or the chromosomes responsible for them, are transferred to the progeny together, with the aid of an as yet unknown mechanism, which helps to preserve genotypes that have become adapted in the course of micro- and macroevolution.

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Book review

D. PIMENTEL (Ed.): Encyclopedia of Pest Management. Marcel Dekker, Inc., New York, Basel, 2002. 929 pp. ISBN 0-8247-0632-0

There can be little doubt that the fight against pests remains one of the major challenges facing agriculture. Although the use of chemical pesticides has become general in crop production since the Green Revolution, diseases still destroy an incredibly high proportion of the crop. For the eight plant species grown on the largest areas, insects are responsible for yield losses of 15%, fungi and weeds each destroy around 13%, while a further 10% is lost during storage following harvest. The damage can be estimated at around $244 billion a year. More and more frequently reports are given of epidemics among livestock, many of which have serious consequences for humans.

The team of contributors led by Professor David Pimentel of Cornell University undertook the challenging task of providing the reader with a comprehensive picture of the results of research on pest management. A total of 289 experts from 18 countries have written 265 brief reports, each of around 2–4 pages, summarising their particular field of research. The reader is supplied with a wide range of knowledge on various types of pesticides, their action mechanisms, fields of utilisation, target organisms, and application technologies. The book also contains information on production techniques aimed at the reduction or elimination of pesticide use, ranging from integrated pest management to organic farming. A broad spectrum of alternative techniques is presented for decimating pathogens. Great emphasis is placed on biological protection and on sustainability, one of the major challenges of our time.

The articles do not only cover pest management as such, since the authors include a large number of experts active in related fields. Information is provided on the human aspects of pest management, the importance of which is underlined by the fact that 26 million people are poisoned each year by pesticides, with around 220,000 fatalities, while the group of mycotoxins also includes many dangerous, carcinogenic compounds. A discussion can be found on the economic damage caused by pests, and how this can be estimated. Nor has the legal control of pest management been forgotten. It is impossible in a brief review to list the multiplicity of topics covered by the book, all of which are directly or indirectly connected with pest management.

The articles are not grouped according to subject matter, but are arranged in alphabetical order of the titles. Any difficulty this may cause is well compensated for by the extremely detailed index at the end of the book, which makes it simple to find articles which could often be classified in several fields of science. The references listed at the end of each article will be of assistance to readers who wish to know more about the given topic.

Due to the multiplicity of fields it covers, this book is a must for both public libraries and agricultural research institutions. It will be of interest to specialists wishing to obtain information on the results achieved in related fields, to university students, and to those working in plant protection and animal hygiene, but will also provide valuable information for farmers and those for whom gardening is a hobby.

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RESPONSE OF *VICIA FABA* PLANTS TO THE INTERACTIVE EFFECT OF SODIUM CHLORIDE SALINITY AND SALICYLIC ACID TREATMENT

A. A. TAMMAM

BOTANY DEPARTMENT, FACULTY OF SCIENCE, ALEXANDRIA UNIVERSITY, ALEXANDRIA, EGYPT

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The leaf area, fresh and dry matter, and water content in the roots and shoots of broad bean were significantly reduced with a rise in salinity. The protein components in the roots and shoots decreased in response to salinity, whereas the proline content significantly increased. The sodium content in both roots and shoots increased with increasing salinity, whereas potassium and calcium decreased. Salinity induced an increase in total amino acids and ammonia.

Spraying with salicylic acid (SA) increased the three growth parameters, stimulated the synthesis of protein at all salinities, retarded the accumulation of proline, retarded the accumulation of Na^+ and increased the content of K^+ and Ca^{2+}. The total content of amino acids was about 1.6-fold the untreated control and there was a drastic increase in the content of threonine and serine.

The electrophoretic pattern of SA-treated seedlings showed 21 polypeptides compared to 12 in the salt-treated ones. Salinity plus SA resulted in the disappearance of 4 polypeptides. In addition, two peptides with molecular masses of 99 and 102 kDa appeared in the gel in both NaCl-treated seedlings and NaCl+SA-treated seedlings.

Key words: amino acids, proline, proteins, mineral contents, NaCl, salicylic acid, *Vicia faba*

Introduction

Salinity is a serious and potential problem on irrigated lands in arid and semiarid zones in many parts of the world. In general, salinity reduces the growth of salt-sensitive glycophytes and affects many aspects of the plant metabolism, inducing several changes in their morphology, as well as in the contents and activities of many enzymes (Greenway and Munns, 1980; Shaddad et al., 1990; Flowers and Yeo, 1992; Marschner, 1995).

Several investigators reported that water stress caused an alteration of gene expression in plants, resulting in inhibition or enhancement of specific protein synthesis (Singh et al., 1989; Skriver and Mundy, 1990; Chang et al., 1996).

Reviron et al. (1992) detected a 22-kDa polypeptide protein in *Brassica napus* plants subjected to water or salt stresses, but the polypeptide disappeared upon rehydration. Singh et al. (1987) and Kononowicz et al. (1992) demonstrated that a 26-kDa protein referred to as osmotin was intimately associated with salt adaptation in cultured tobacco cells. Rey et al. (1998)

reported the accumulation of a 32-kDa polypeptide in water-stressed *Solanum tuberosum*, and they suggested that the polypeptide played a role in the preservation of the redox potential of the chloroplastic protein during water stress. Harrak et al. (2001) reported the accumulation of a 65-kDa protein in the leaves of drought–stressed tomato (*Lycopersicon esculentum* cv. Starfire) plants. The protein content dropped to the control value when the drought-stressed plants were rewatered.

Attempts have been made to employ active phytohormones or vitamins to overcome the drastic effects of salinity on plant growth (Shaddad and El-Tayeb, 1990; Shaddad et al., 1990; Ahmed et al., 2001).

Salicylic acid (SA) has been reported to cause a multitude of effects on the morphology and physiology of plants (Raskin, 1992; Pierpoint, 1994; Pancheva et al., 1996) and to induce a protective mechanism enhancing resistance to biotic and abiotic stresses (Lopez-Delgado et al., 1998). The ameliorating effects of SA on growth and plant metabolism under salt stress are still not well understood. Therefore, the present study was conducted to investigate whether the effects of exogenous application of SA would: i) counteract the adverse effect of salinity on growth, ii) have a beneficial effect on metabolic processes of broad bean plants. It was also hoped to investigate the effects of SA on changes in protein profiles under salinized conditions.

Materials and methods

Vicia faba L. cv. Giza Blanka was grown in plastic pots containing 300 g of garden soil and peat moss (1:1; v/v). The pots were placed in the laboratory under natural light. The temperature varied from 20–24°C during the day and 15–19°C during the night and the pots were irrigated to about 90% of water holding capacity. Salinity treatment was carried out by adding 0–20–40–60–80–100 mM NaCl to the soil mixture before irrigation. After two weeks the salinized and non-salinized plants were sprayed with 10 cm^3 of 200 μmol SA. After four days, the treatment was repeated with 20 cm^3 of SA. Samples were taken for fresh matter determination when the plants were 25 days old. Dry matter was determined after drying the plants to constant mass in an aerated oven at 70°C. Leaf area was determined with a planimeter (SOKKIA planimeter KP–90 UK). Soluble and total protein contents were determined according to Hartree (1972). Ammonia and free individual amino acids were estimated according to Speckman et al. (1958). Proline content was determined according to Bates et al. (1973). The mixed acid digestion method of Allen et al. (1974) was used to prepare samples for the determination of minerals. Sodium, potassium and calcium concentrations were estimated using a flame photometer (CORN NG 400). All determinations were done in duplicate and three plants were taken for each determination.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous buffer system described by Laemmli (1970) and modified by Hames and Rickwood (1990). Molecular masses were determined according to Weber and Osborn (1969).

Results

The leaf area, fresh and dry matter, and water content of broad bean plants were significantly reduced by a rise in salinity (Table 1). Spraying with 200 μmol SA resulted in an increase in the three parameters, whatever the salinization level used.

Salinity also induced a marked and progressive decrease in protein level both in the roots and shoots of the broad bean plants. Spraying with SA strongly stimulated protein content in the roots and shoots of the control plants, as well as in the salinized ones (Table 2). Proline was significantly increased in response to salinization in the shoots and roots of bean plants; this increase was greater in the roots than in the shoots. SA inhibited the accumulation of proline in the saline plants, but could not normalize it (Table 3).

The sodium content in both the shoots and roots of the variously salinized plants increased with increasing salinity. However, at severe salinity levels, the accumulation of sodium in the shoots was markedly higher than in the roots. Salinity also reduced the content of potassium and calcium in the shoots and roots.

Treatment with SA retarded the accumulation of Na^+, particularly in the shoots, and resulted in a marked and progressive increase in K^+ and Ca^{2+} contents as compared with those of the corresponding salinized plants (Table 4).

Salinity resulted in an approximately 12% increase in total free amino acid and a marked increase in ammonia (to about 10 times the control value). The latter was greatly reduced by SA treatment. The amount of free amino acids in bean plants exposed to 100 mM NaCl and treated with 200 μ mol SA was approximately 1.6-fold that of the untreated control.

The dominant amino acids in untreated, salinized and SA-treated plants were leucine, threonine and serine. In the salinization and SA treatments, cystine was only present in traces. Treatment of salinized bean plants with SA induced a dramatic increase in threonine and serine to approximately 2.7-fold the control values (Table 5).

There were pronounced differences in the electrophoretic patterns of proteins in bean seedlings under the four different treatments (Fig. 1). The control samples showed about 17 peptides with molecular masses ranging between 12.5 and 84.5 kDa. The pattern of SA-treated seedlings showed 21 polypeptides within the same range. Salt-treated seedlings showed 12 polypeptides; those of 26, 18, 14 and 12 kDa had high band intensity.

The interaction between salinity and SA resulted in the appearance of 8 polypeptides. Those with molecular masses of 16, 22, 32 and 43 kDa were not detected on the gel compared to NaCl-treated plants (lane 2), while the 28, 26, 18, 14 and 12 kDa polypeptides gave a denser band compared to the control. In addition, two peptides (99 and 102 kDa) appeared in the gel in both the salt-treated seedlings (lane 2) and the NaCl+SA-treated seedlings (lane 4).

Table 1

Changes in the fresh matter, dry matter (g $plant^{-1}$) and leaf area (cm^2 $plant^{-1}$) of 25-d-old broad bean seedlings in response to salt stress and salicylic acid treatment

Treatment	Roots			Shoots			
	Fresh matter	Dry matter	Water content %	Fresh matter	Dry matter	Water content %	Leaf area
Control	1.43	0.19	84.5	2.65	0.26	90.2	19.73
20 mM NaCl	1.26**	0.18	84.3	2.64	0.25	90.5	17.92
40 mM NaCl	0.63**	0.11**	82.5	1.50**	0.21*	86.0	16.56
60 mM NaCl	0.32**	0.08**	75.0	1.00**	0.18**	82.0	14.36*
80 mM NaCl	0.23**	0.07**	69.5	0.79**	0.14**	82.0	12.06**
100 mM NaCl	0.13**	0.05**	61.5	0.37**	0.13**	65.0	10.14**
Control + SA	1.89**	0.22**	88.3	2.98**	0.28	91.0	22.22
20 mM NaCl+SA	1.88**	0.22**	88.2	2.61	0.26	90.2	20.85
40 mM NaCl+SA	1.45**	0.20	86.2	2.44*	0.24	90.1	18.40
60 mM NaCl+SA	1.02**	0.15	85.2	2.02**	0.23	89.0	17.0
80 mM NaCl+SA	0.83**	0.12**	85.0	1.92**	0.21**	89.0	15.8
100 mM NaCl+SA	0.75**	0.11**	85.0	1.33**	0.18**	86.4	15.2*
$LSD_{5\%}$	0.045	0.028		0.203	0.027		4.301
$LSD_{1\%}$	0.075	0.047		0.337	0.046		6.045

*, ** Significant at the 5% and 1% level of probability compared with the absolute control

Table 2

Changes in the content of soluble and insoluble proteins [mg g^{-1} (d.m.)] of 25-d-old broad bean seedlings in response to salt stress and salicylic acid treatment

Treatment	Roots			Shoots		
	Sol-P	Insol-P	Total	Sol-P	Insol-P	Total
Control	112.50	77.54	190.04	137.30	82.20	219.50
20 mM NaCl	102.92	77.08	180.03	117.04**	87.01	204.05*
40 mM NaCl	90.50*	34.61**	125.11**	120.50**	58.41**	178.91**
60 mM NaCl	83.69**	24.98**	108.67**	99.61**	36.89**	136.50**
80 mM NaCl	70.39**	17.99**	88.38**	69.89**	25.33**	95.22**
100 mM NaCl	50.00**	10.90**	60.90**	73.78**	12.83**	89.61**
Control + SA	126.87*	90.80*	217.67**	140.75	104.71**	245.46**
20 mM NaCl+SA	120.75	90.60*	211.35**	140.17**	101.83**	242.00**
40 mM NaCl+SA	110.03	80.64*	190.67*	122.89**	81.86	204.75*
60 mM NaCl+SA	110.21	70.42	180.63	116.80**	76.70	193.50**
80 mM NaCl+SA	100.33	70.35	170.68*	110.17**	61.20**	171.37**
100 mM NaCl+SA	94.71**	43.20**	137.91**	110.49**	40.59**	151.08**
$LSD_{5\%}$	17.555	13.004	14.683	9.166	11.418	15.035
$LSD_{1\%}$	29.116	21.568	24.351	15.202	18.938	24.936

*, ** Significant at the 5% and 1% level of probability compared with the absolute control

Table 3

Changes in the proline content [μg g^{-1} (f.m.)] in the roots and shoots of 25-d-old broad bean seedlings in response to salt stress and salicylic acid treatment

Treatment	Roots	Shoots
Control	0.038	0.053
20 mM NaCl	0.099	0.119
40 mM NaCl	0.223	0.291
60 mM NaCl	0.570	0.530**
80 mM NaCl	1.625**	0.655**
100 mM NaCl	1.575**	1.220**
Control + SA	0.032	0.041
20 mM NaCl+SA	0.043	0.057
40 mM NaCl+SA	0.187	0.145
60 mM NaCl+SA	0.284	0.147
80 mM NaCl+SA	0.456*	0.219
100 mM NaCl+SA	0.550**	0.411*
$LSD_{5\%}$	0.474	0.318
$LSD_{1\%}$	0.318	0.528

*, ** Significant at the 5% and 1% level of probability compared with the absolute control

Table 4

Changes in the mineral contents [mg g^{-1} (d.m.)] in the roots and shoots of 25-d-old broad bean seedlings in response to salt stress and salicylic acid treatment

Treatment	Roots			Shoots		
	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}
Control	1.1	18.6	12.4	0.7	26.8	15.5
20 mM NaCl	1.5	17.0	11.6	1.2	25.9	16.1
40 mM NaCl	9.4**	10.3**	9.3	10.5**	24.2	10.9
60 mM NaCl	12.8**	10.3**	6.2*	13.3**	14.3**	10.7
80 mM NaCl	19.8**	8.5**	6.7*	18.8**	10.5**	9.3*
100 mM NaCl	21.2**	7.6**	5.4*	24.9**	5.8**	7.5*
Control + SA	0.9	19.0	13.1	0.9	30.9	18.5
20 mM NaCl+SA	1.4	18.8	14.7	1.1	26.1	18.8
40 mM NaCl+SA	5.6**	17.9	11.3	4.5	27.6	17.9
60 mM NaCl+SA	5.5**	11.9*	11.5	9.1**	20.6	13.4
80 mM NaCl+SA	13.9**	9.4**	8.1	16.2**	16.2**	13.1
100 mM NaCl+SA	19.3**	8.1**	6.6*	20.4**	14.3**	9.5*
$LSD_{5\%}$	3.950	6.253	5.299	4.556	6.495	6.615
$LSD_{1\%}$	6.551	10.372	8.788	7.557	10.773	10.972

*, ** Significant at the 5% and 1% level of probability compared with the absolute control

Table 5
Changes in the contents of ammonia and amino acids [mg g^{-1} (f. m.)] in 25-d-old broad bean seedlings in response to salt stress and salicylic acid treatment

Treatment	Control	100 mM NaCl	Control+SA	100m M NaCl+SA
Alanine	7.83	11.12	10.81	10.47
Glycine	1.15	2.82	1.23	0.94
Isoleucine	13.97	18.53	15.11	17.97
Leucine	62.72	74.32	75.15	63.93
Threonine	62.42	64.19	55.27	174.75
Valine	15.29	19.02	17.29	19.25
Aspartic acid	13.31	15.29	14.90	20.06
Glutamic acid	25.24	28.84	25.24	33.35
Arginine	14.43	13.68	0.92	11.78
Histidine	31.21	24.33	25.83	33.88
Lysine	8.65	17.20	21.36	11.46
Phenylalanin	13.90	12.34	12.00	18.67
Tyrosine	13.18	15.19	19.45	15.63
Methionine	3.17	4.04	4.12	3.01
Cystine	9.82	0.42	0.42	0.67
Serine	46.91	48.25	41.55	131.36
Total	343.19	381.92	340.65	557.18
Ammonia	1.72	16.90	1.95	1.84

*, ** Significant at the 5% and 1% level of probability compared with the absolute control

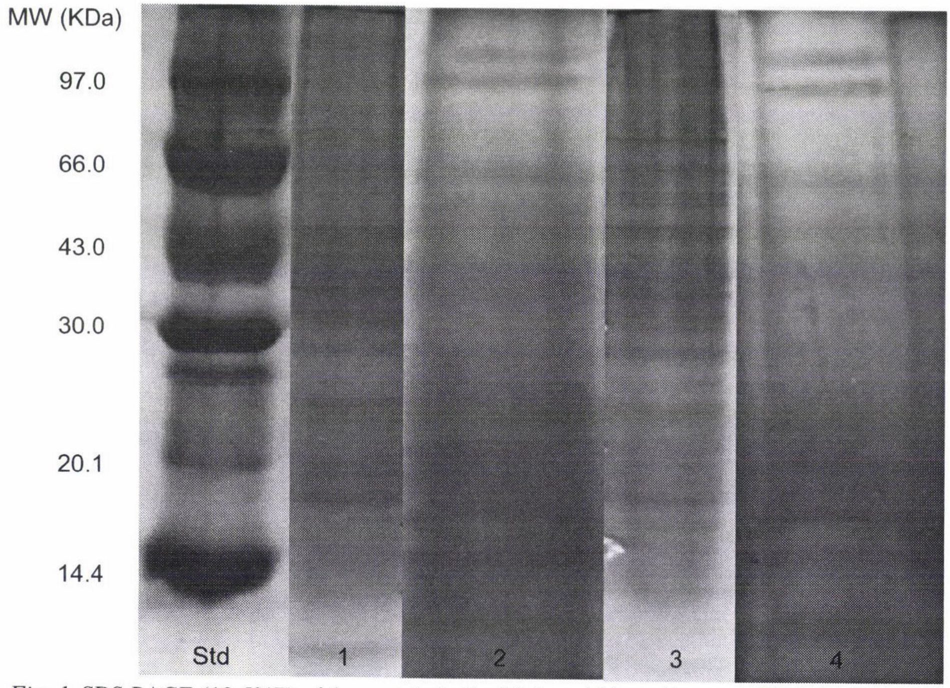

Fig. 1. SDS-PAGE (12.5%T) of the protein in the 25-day-old broad bean seedlings in response to salt stress and salicylic acid treatment. Anode is towards the bottom of the photo. 1: Control, 2: 100mM NaCl, 3: Control + SA, 4: 100 mM NaCl + SA

Discussion

Salinity induced a significant decrease in the leaf area, fresh matter, dry matter and water content of broad bean plants, which is in agreement with the results reported for many plants including *Zea mays*, *Triticum aestivum*, *Vicia faba* and *Argyranthemum coronopiforlium* (Zidan et al., 1990; Shalaby et al., 1993; Cordovilla et al., 1994; Morales et al., 1998). These adverse effects of salinity were reported to be due to sodium toxicity (Lutts et al., 1996) and/or water deficit (Greenway and Munns, 1980).

The inhibitory effect of salinity was located in the roots, judging by the markedly more pronounced inhibition of their dry mass compared to the shoots. This could greatly reduce the high absorbing zone, leading in turn to the limitation of nutrient uptake and the suppression of water status in salt-affected broad bean plants (Marcelis and van Hooijdonk, 1999). This seems to be closely associated with the marked accumulation of ammonia in salt-treated plants (to about 10 times the control), resulting in a drastic disturbance in growth building materials (structural proteins) and osmoprotectants (soluble protein and amino acids).

Spraying the salinized plants with SA induced an increase in dry mass production as compared with the corresponding salinized plants. It could be concluded that the marked and progressive increase in dry mass production in the roots of bean plants treated with SA enhanced the absorption and translocation of water, thus maintaining the tissue water content at around the level of the absolute control even in plants grown under severe salinity (Table 1). This was usually accompanied by a pronounced increase in the leaf area of SA-treated bean plants. Marcelis and van Hooijdonk (1999) reported that 80% of the salinity effect on plant growth was likely to be the result of a reduction in leaf area, which could lead to the suppression of photosynthesis.

Treatment with SA significantly reduced transpiration in kidney bean (*Phaseolus vulgaris*) leaves (Larque-Saavedra, 1978; Barkosky and Einhellig, 1993), which means that SA treatment could increase the efficiency of plant water uptake, conservation and utilization, maintaining the tissue water content at around the normal value even at severe salinity (i.e. improvement of plant water status).

Salinity induced a significant decrease in soluble and total protein contents, while the amino acids, including proline, were significantly increased in bean plants. This could be due to the inhibition of the incorporation of amino acids into proteins in NaCl treatments (Handa et al., 1983). The accumulation of amino acids could play a role in osmotic adjustment and serve as a source of carbon and nitrogen (Ayoub et al., 1992; Bolarin et al., 1995; Chiang and Dandekar, 1995; Clifford et al., 1998).

The marked increase in total protein and proline in SA-treated plants indicates that SA might alleviate the imposed salt stress either via osmotic adjustment or by conferring desiccation resistance to plants due to increasing water content. In addition, proline also serves as a sink for energy to regulate redox potential (Blum and Ebercon, 1976), as a hydroxy radical scavenger (Smirnoff and Cumbes, 1989), as a solute that protects marcromolecules against denaturation (Schobert and Tschesche, 1978) and as a means of reducing the acidity in the cell (Venekamp, 1989).

Salt treatment of broad bean seedlings resulted in the disappearance of five polypeptides, while the peptides with molecular masses of 26, 18, 14 and 12 kDa increased in their intensity and two peptides (99 and 102 kDa) appeared on the gel. Reviron et al. (1992) detected a 22-kDa protein in *Brassica napus* plants subjected to water and salt stresses which disappeared upon rehydration. Gupta (1999) identified two polypeptides with molecular masses of 60 and 18 kDa in selected embryos and plants of salt-treated orchard grass. Rey et al. (1998) reported the accumulation of a 32-kDa polypeptide in water-stressed *Solanum tuberosum*. They suggested that the polypeptide played a role in preserving the redox potential of chloroplastic proteins during water deficit. However, spraying broad bean seedlings with 200 μmol SA induced the appearance of two peptides with molecular masses of 99 and 102 kDa and increased the intensity of the 28, 26, 18 and 14 kDa polypeptides. Silhavy et al. (1995) reported that the treatment of detached *Solanum chacoense* leaves with SA resulted in a slight accumulation of water stress-inducible genes which encode a putative protein starting with 20 amino acids. Chao et al. (1999) reported that SA induced an increase in leucine amino peptidase RNAs in response to salinity and water deficit in tomato (*Lycopersicon esculentum*).

Collectively the results obtained in this work demonstrate the importance of the exogenous application of salicylic acid in counteracting the inhibitory effect of salinity on growth criteria, which was associated with an enhancement of the production of proteins (building materials) and also improved osmoregulation by increasing phytosolutes (soluble proteins and amino acids.) Additionally, SA lowered Na^+ and enhanced K^+ accumulation; consequently the K^+/Na^+ ratio was higher in SA-treated plants.

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EFFECT OF SOWING DATE ON YIELD AND YIELD COMPONENTS OF THREE OILSEED RAPE VARIETIES

G. FATHI[1], S. A. SIADAT[1] and S. S. HEMAIATY[2]

[1]RAMIN AGRICULTURAL RESEARCH AND EDUCATIONAL CENTER, SHAHID CHAMRAN UNIVERSITY, AHWAZ, IRAN
[2]ARDABIL AGRICULTURAL RESEARCH STATION, ARDABIL, IRAN

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In order to elucidate the effect of sowing date on the yield and yield components of oilseed rape (*Brassica napus* L.), three varieties – Tower, Rafal and Global – were sown from 7 November to 22 December 2000-01 on four dates at an interval of 15 days in the Dezful region of Iran. A split-plot design based on randomized complete blocks with four replications was used in the experiment, where the sowing dates and cultivars formed the main and subplots, respectively. Variables including plant height, axillary branches/plant, pods/plant, seeds/pod, single seed weight, biomass, seed oil content and seed yield were measured. The soil of the experimental site had a loamy clay texture. The site had 250 mm annual precipitation and was located in the semi-arid zone.

The results showed that the sowing date had a highly significant effect on morphological characteristics, yield components, oilseed rape yield and seed oil content. A delay in the sowing date caused a reduction in all the yield components especially in pods/plant, and in oilseed yield, which dropped from 285 g m^{-2} when sown on 7 November to 135 g m^{-2} when sowing was delayed to 22 December. Variations in sowing dates had different effects on the individual yield components, with pods/plant, seeds/pod and single seed weight decreasing to the greatest extent. The significant effect of variety on all characters with the exception of single seed weight indicated that there were genetic differences between the studied cultivars. Oilseed yield showed significantly positive correlations with pods/plant (r=0.93), single seed weight (r=0.83) and seeds/pod (r=0.66). The results of path analysis showed that pods/plant and seeds/pod had the highest positive and negative effects on oilseed yield, respectively. Finally, considering the susceptibility of pods/plant to variations in sowing date and the importance of this character in the size of the oilseed yield, a delay in the planting date in the Dezful region was found to reduce the oilseed yield through a reduction in pods/plant.

Key words: oilseed rape, sowing date, yield components, correlation and path analysis

Introduction

The determination of suitable planting dates plays an important role in the conformation of plant growth stages to desirable environmental conditions, resulting in maximum yields. According to Thurling (1974) planting date had a considerable effect on the seed yield through its influence on the yield components, as late planting decreased the secondary branches/plant and pods/plant and finally caused an intense drop in seed yield. The late sowing of rapeseed decreased seed yield through the synchronization of the pod-filling period with high temperatures, a reduction in assimilate production, the occurrence of drought stress, a shortening of the pod-filling period and the acceleration of plant maturity (Mendham et al., 1981). In fact, the early sowing of winter rapeseed is the most important method for increasing the seed and oil yield (Mendham and Shipway, 1981). In the studies of Taylor and Smith (1992) late sowing caused a decrease in biomass, harvest index and seed oil content. Late sowing also reduced the seeds and pods/square metre and the thousand-seed weight, though the response of seeds/pod to changes in sowing date was different. Whitefield (1992) reported that the reduction in the seed yield due to late planting was the result of the increasing respiration rate of the pods, losses in assimilates and a rise in the empty seed percentage. The economic yield of late-sown plants was decreased by a reduction in the harvest index, emerged plants per unit of area and axillary branches per plant (Scarisbrick et al., 1982). Thurling (1974) showed that an unsuitable planting date reduced the number of pod-bearing axillary branches, seed yield and thousand-seed weight, but had a negligible effect on seeds/pod. He found that the response of seed yield to late sowing dates depended on the interrelationship between seeds/pod and number of pods per unit area.

In regions with early cold weather, the limiting effects of late planting were more considerable (Mendham and Scott, 1975). In these regions, the late planting of winter rapeseed produced short stems, reduced the branching and green area of the plants, and finally caused a reduction in assimilate production in the pod stage, decreasing the pods/plant by 60% (Mendham and Shipway, 1981). Hodgson (1979) showed that the oil content of the seeds was reduced by delays in planting date from April to September in Australia. In all, the oil content of the seed was mainly determined by the climatic conditions, variety and planting date; late planting decreased the oil content and the oil yield (Jasinska, 1987).

This study was carried out in order to study interactions between environment and variety with the aim of identifying varieties adapted to the environmental conditions in the Dezful region of Iran and evaluating the best planting date for maximum seed and oil yield.

Materials and methods

The experiment was carried out during 2000-01 in the Sibli region of Dezful, Iran (32° 16' N, 45° 25' E, 82 m above sea level). The experimental site had a hot climate (35.5°C) with a dry, hot summer. The region is located in the semi-arid zone and has an annual precipitation of 250 mm. The soil of the experimental site was loamy clay in texture, with pH 7.6, bulk density 1.4 g/cm^3, low nitrogen (6.3 ppm), moderate phosphate (9.5 ppm) and medium potash (142.5 ppm). It was low in organic carbon (0.78%). The electrical conductivity of the soil was 0.65 mmhos/cm. The experiment was arranged in split-plots based on a random complete block design with planting dates (7 November, 22 November, 7 December and 22 December) in the main plots and varieties (Tower, Rafal and Global) as sub-plots with four replications. The seeds were sown after soil preparation in autumn with the agrotechnological practices usual at the Dezful agricultural research centre.

Most of the annual precipitation fell between November and the end of April. The trial site, which had been used for wheat production in the preceding year, was cultivated in autumn 1999. Urea fertilizer (46% nitrogen) was applied to the soil surface at a rate of 150 kg/ha, with a basal dose of 50 kg nitrogen and 40 kg P_2O_5/ha at the time of sowing, mixed with the soil using a disc harrow, and another 100 kg nitrogen top-dressed in 2 equal splits at the time of stem elongation and flowering. The crop was sown at 50 cm row spacing using 5 kg/ha seed. At seeding, two seeds were planted in each hill, but at the 3–4-leaf stage the emerged seeds were thinned to achieve one plant per hill.

Until crop establishment, preliminary irrigation was performed once every 4–6 days. Afterwards, until the end of the crop growing period, the plots were irrigated whenever it was necessary. Hand weeding was performed frequently during the crop growing period.

During the growing season, plant height, axillary branches/plant, pods/plant, seeds/pod and single seed weight were measured on five randomly selected plants per plot. The plants were harvested on 4 m^2 of plots on 8–18 May 1999. After harvesting, the seed yield (Y_r) and its moisture content (WC) were measured and the, standard yield (Y) with 12% moisture was estimated according to the following formula:

$$Y=Y_r \times [(100-WC)/88]$$

In addition, the seed oil content was determined by the Soxhlet method. Analysis of variance and means comparison were carried out with the MSTATC statistical program and graphs were drawn using EXCEL MS-OFFICE software.

Results and discussion

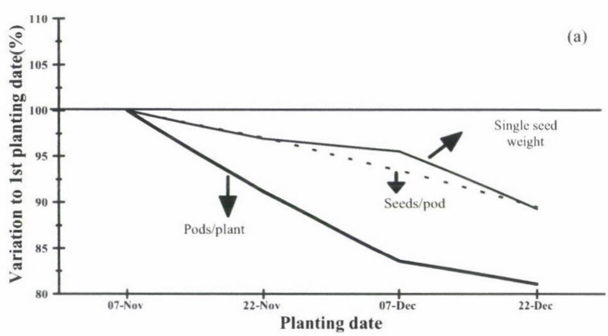
(A) Yield components

Delaying the planting date caused significant ($p<0.01$) decreases in pods/plant, seeds/pod and single seed weight (Table 1). Reductions in pods/plant and single seed weight are common effects of delays in planting (Allen et al., 1971; Mendham and Shipway, 1981; Pechan and Morgan, 1985; Scarisbrick et al., 1982), but seeds/pod gives different responses to variations in planting date (Scarisbrick et al., 1982). In the present study, the intensity of variation in each yield component due to delays in planting were differentiated, and pods/plant, seeds/pod and single seed weight showed the greatest responses (Fig. 1a). The importance of pods/plant and the susceptibility of this parameter to environmental conditions have been emphasised by other scientists (Thurling, 1974; Norton and Bilsborrow, 1991). The differences between varieties from the viewpoint of pods/plant and seeds/pod were highly significant ($p<0.01$), but for single seed weight the difference was non-significant (Table 1). Variety Tower was ranked highest with 302 pods/plant, 24 seeds/pod and 3.6 mg single seed weight (Table 1). The coefficients of correlation estimated between seed yield and yield components showed that pods/plant ($r=0.93$), single seed weight ($r=0.83$) and seeds/pod ($r=0.66$) had the highest correlation with seed yield (Table 2 and Fig. 2) (Taylor and Smith, 1992; Thurling, 1974).

Table 1
Effects of planting date and variety on rapeseed yield, yield components, biomass, harvest index and oil percentage

	Means of squares						
Planting dates	1⁺	2	3	4	5	6	7
7 November	331.0a*	25.3a	3.76a	285.2a	1050.83a	27.1a	41.5a
22 November	301.7b	24.6ab	3.64b	236.7b	1011.42b	23.4b	41.0a
7 December	276.7c	23.7bc	3.59c	160.0c	904.10c	17.7c	39.9b
22 December	268.3c	22.7c	3.36d	135.6d	818.06d	16.6d	38.8c
LSD (P=0.05)	15.0	1.18	0.05	14.7	30.23	0.72	0.79
Variety							
Tower	302.5a	24.7a	3.60a	217.8a	973.4a	21.9a	40.5a
Rafal	287.5b	23.2b	3.58a	204.9b	915.5c	21.9a	40.5a
Global	293.2b	24.2a	3.59a	190.4c	949.4b	19.7b	40.0b
LSD (P=0.05)	7.4	0.64	0.03	4.62	10.4	0.26	0.38

⁺1: Pods/plant; 2: Seeds/pod; 3: Single seed weight (mg): 4: Seed yield (g/m^2); 5: Biomass (g/m^2); 6: Harvest index (%); 7: Oil content (%); *Means followed by the same letter are not significantly different at the P=0.05 level according to Duncan's multiple range test.



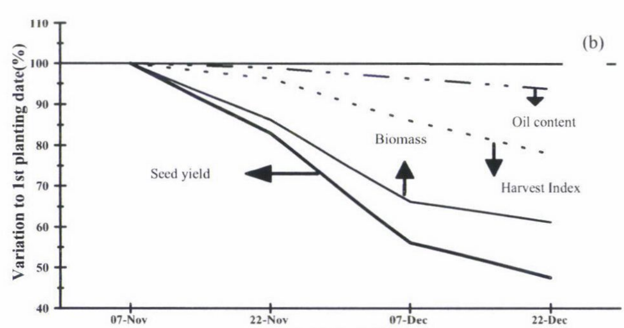


Fig. 1. Effect of planting date on (a) yield components and (b) seed yield, biomass, harvest index and oil content of rape seed

(B) Biomass and harvest index (HI)

The biomass and HI were significantly ($p<0.01$) reduced by late planting dates (Table 1). These results were in accordance with other studies (Rao and Mendham, 1991; Taylor and Smith, 1992; Whitefield, 1992). Scarisbrick et al. (1982) showed that HI reduction due to delayed sowing was one of the most important factors in the reduction in seed yield. The delay in planting decreased the efficiency of assimilate transfer to the the grains, causing a reduction in seed yield (Scarisbrick and Daniels, 1986). The studied varieties showed differences ($p<0.01$) in biomass and HI (Table 1), with the variety Tower producing the highest biomass (973 g/m^2) and HI (21%).

(C) Seed yield and seed oil content

The results of this study showed that seed yield was significantly ($p<0.01$) decreased by a delay in planting (Table 1). Indeed, 15, 30 and 45 days delay from 7 November reduced the seed yield from 285 g/m^2 to 236, 160 and 135 g/m^2, respectively (Table 1). This result was in accordance with other studies (Loof, 1960; Thurling, 1974; Mendham and Scott, 1981; Scarisbrick et al., 1982; Taylor and Smith, 1992). Significant ($p<0.01$) differences were observed between the varieties, which had yields of 217 (Tower), 204 (Rafal) and 190 g/m^2 (Global) (Table 1). The significant ($p<0.01$) differences between planting date × variety interaction effects were confirmed by various responses of varieties via planting date variations. In other words, delaying planting caused a reduction in rapeseed yield. The severe decreases in seed yield observed for Global showed its high susceptibility and late-maturing nature.

The effects of the studied sowing dates and varieties on the seed oil content were significant (Table 1). A decrease in rapeseed oil content due to a delay in planting has been reported by other workers (Thurling, 1974; Pechan and Morgan, 1985; Jasinska, 1987). The response of rapeseed oil content to changes in planting date was low, indicating that this character was controlled by the genotype, rather than the environmental conditions, although at the latest planting date, due to the severe reduction in seed yield, the oil yield was reduced substantially (Loof, 1960; Thurling, 1974; Hodgson, 1979; Pechan and Morgan, 1985; Jasinkska, 1987; Taylor and Smith, 1992).

(D) Path analysis

Pods/plant (1.248) and seeds/pod (–0.107) had the highest positive and negative direct effects on seed yield. Pods/plant had the greatest indirect effects on seed yield via branches/plant (1.217), biomass (1.167) and plant height (1.166). Thurling (1974) reported that the rapeseed yield potential was positively correlated with the growth period and vegetative growth, so the timely planting of rapeseed led to high seed yields by prolonging the growth period, giving a proportional increase in the flowers and pods per plant. Finally, due to the high succeptibility of pods/plant to planting date variations (Fig. 1a) and the most important role of this character in determining the rapeseed yield (Fig. 2a and Table 2), the results of this study showed that delaying planting decreased the rapeseed yield in the Dezful region through a reduction in pods/plant and also indicated that pods/plant could be a useful character in rapeseed selection programmes.

Table 2
Path analysis coefficients of related characters with seed yield

Character	Direct effect	Indirect effect via: Plant height	Branches/plant	Pods/plant	Seeds/pod	Seed weight	Biomass	Total
Plant height	−0.008	–	−0.007	−0.008	−0.007	−0.006	−0.007	0.887
Branches/plant	−0.342	−0.309	–	−0.334	−0.271	−0.290	−0.301	0.927
Pods/plant	1.248	1.166	1.217	–	0.987	1.064	1.167	0.966
Seeds/pod	−0.107	−0.090	−0.085	−0.085	–	−0.084	−0.087	0.735
Seed weight	0.211	0.163	0.179	0.180	0.165	–	0.195	0.859
Biomass	−0.042	−0.038	−0.037	−0.039	−0.034	−0.039	–	0.927

Residual effect = 0.227

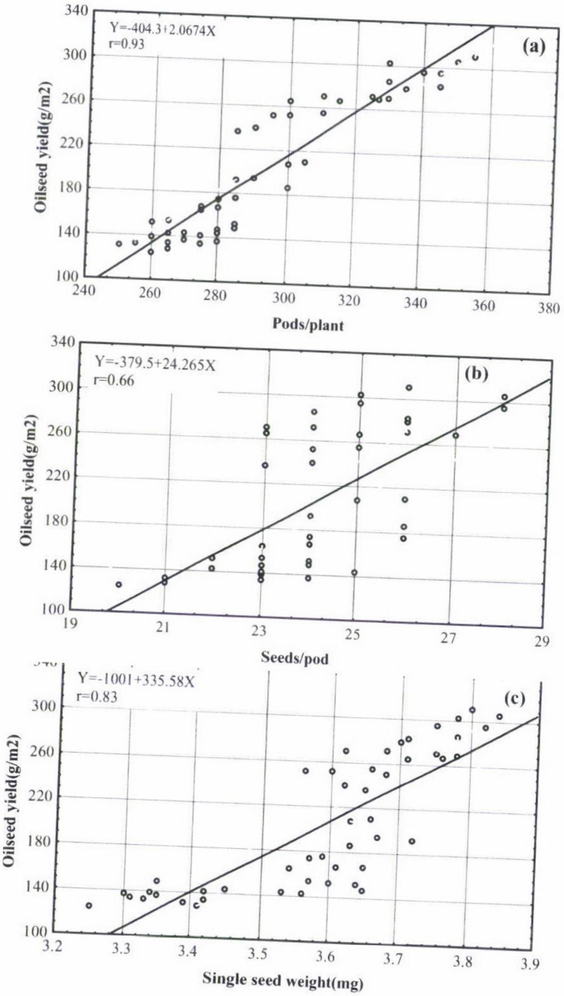


Fig. 2. Correlations between oilseed yield and (a) pods/plant, (b) seeds/pod and (c) single seed weight

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APPLICATION OF MORPHOLOGICAL DESCRIPTIONS AND GENETIC MARKERS TO ANALYSE POLYMORPHISM AND GENETIC RELATIONSHIPS IN MAIZE (*ZEA MAYS* L.)

E. NAGY[1], G. GYULAI[2], Z. SZABÓ[2], Z. HEGYI[1] and L. C. MARTON[1]

[1]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY
[2]DEPARTMENT OF GENETICS AND PLANT BREEDING, SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY

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Studies involving morphological description with both dominant (RAPD) and codominant (SSR, isoenzyme) molecular markers were made on 28 maize inbred lines of known genetic background with a final aim of prediction of heterosis. The genetic distance and degree of relationship between the lines was determined using cluster analysis. Only a very limited extent of allele polymorphism could be detected in isoenzyme analyses as the 28 lines formed only 16 gel electrophoretic groups, indicating that certain lines had identical isoenzyme patterns. On the basis of RAPD and gene-specific microsatellite (SSR) markers, however, all the lines could be distinguished from each other. When the lines were grouped according to genetic background it was found that although the individual marker systems only partially reflected the actual relationships between the lines, a joint processing of the data, supplemented with morphological data, revealed a close correlation between the groups formed on the dendrogram and the genetic background.

Key words: maize, polymorphism, isoenzyme, RAPD, SSR, cluster analysis

Introduction

The UPOV guidelines elaborated for the registration and patenting of maize varieties are based on the distinctness, uniformity and stability (DUS) of the varieties, but a description based on morphological traits is often not sufficient to satisfy these conditions. One of the main fields where the detection of differences between varieties is required is the establishment of intellectual property rights, so it is essential to use techniques which allow the varieties to be distinguished accurately and reliably (Smith and Smith, 1989).

The rapid development of molecular biological and genetic methods has led to their widespread use in various fields of plant breeding, including maize breeding. In addition to phenotypic traits and the DUS traits based on these (UPOV), the use of molecular markers to analyse polymorphism between breeding stocks facilitates an inventory of genetic stocks, the determination of genetic variability and genetic distances, and variety descriptions for identification and patenting purposes (Smith and Senior, 1999). Identification techniques based on molecular markers (MAS, marker-assisted selection) form three main groups, depending on whether they are based on isoenzyme (Markert and Moller, 1959), RFLP (Botstein et al., 1980) or PCR (Mullis and Faloona, 1987) analyses.

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The UPOV regulations now make it compulsory to carry out starch gel electrophoresis to identify the isoenzyme patterns of maize varieties, based in most cases on the enzymes of the citrate and pentose phosphate cycles (Bourgoin-Greneche and Lallemand, 1993). Although in some cases the isoenzymes possess several loci, with several alleles at each locus, they nevertheless represent only a fraction of the whole genome, so the information obtained in these analyses often falls short of that required from a full genetic analysis. The practical application of isoenzyme polymorphism is also limited by the fact that certain alleles occur with considerably greater frequency than others, while the prediction of heterosis is impossible on the basis of allele polymorphism (Hunter and Kannenberg, 1971; Price et al., 1986; Lamkey et al., 1987).

Among the PCR-based methods developed more recently than the isoenzyme and RFLP analyses, the RAPD (Williams et al., 1990), SSR (Gupta et al., 1994), ISSR (Zietkiewicz et al., 1994) and ISJ-PCR (Weining and Langridge, 1991) methods have become most widely used. The most detailed measurements required for the PCR analysis of maize samples were carried out by Fu et al. (1997).

The grouping of maize varieties into related groups is of great importance in breeding, since genetic distance between the parental lines is a basic criterion for heterosis breeding. The more accurate the description of the maize lines and the demonstration of fine differences between them, the more successfully they can be placed in related groups. Molecular techniques which, directly or indirectly, map the genetic background of individual plants are indispensable for this work (Smith et al., 1997; Pejic et al., 1998).

In the course of the present studies, in addition to morphological description and isoenzyme analysis, 28 maize inbred lines with known genetic background were analysed using PCR-based techniques involving RAPD and gene-linked microsatellite (SSR) markers. The aim of the work was to determine how efficiently and in what combinations these marker systems could be used to demonstrate genetic polymorphism between maize inbred lines and to map relationships between the lines.

Materials and methods

Plant material: 28 maize inbred lines with known genetic backgrounds were used in the study (Table 1).

Morphological description: Maize lines were sown, one to a row, in small-plot experiments set up in a random block design with four replications at two locations in Hungary in two years. The scored traits were determined from the mean of 10 plants per plot, as described by UPOV TG 2/6 (1994) and Smith and Smith (1989).

Isoenzyme analysis: The seeds were germinated under standard conditions in the dark at 25°C in a growth chamber. For isoenzyme analysis (Goodman and Stuber, 1983; Stuber et al., 1988) the coleoptiles of 5-day-old etiolated seedlings were homogenised for approx. 1 min in 30 μl/sample of a solution containing 16.7% sucrose and 8.3% Na ascorbate and then centrifuged for 3 min at 11,000 rpm. Ten μl of the supernatant was applied to a 1 cm gel containing 12.5% starch, which was cut into 1 mm slices after electrophoresis and stained substrate-specifically for the various enzymes. The enzymes examined were: MDH (maleic acid dehydrogenase, 6 loci), IDH (isocitrate dehydrogenase, 2 loci), PGD (6-phosphogluconate dehydrogenase, 2 loci), PGI (phosphoglucose isomerase, 1 locus), PGM (phosphoglucomutase, 2 loci), ACP (acidic phosphatase, 1 locus) and ADH (alcohol dehydrogenase, 1 locus).

Table 1.
Origin of the tested lines

Line		Pedigree
1.	CM 105	CMV 3 × B 14(2)
2.	CM 108	(B 14 × CMV 3)B14
3.	B 14	ISSS
4.	A 632	(MT 42 × B 14)B 14(3)
5.	A 635	(ND 302 × B 14)B 14selfB 14(2)
6.	Mv L2	Pool Dent B 14
7.	CM 7	W 85 × CMV 3
8.	CM 7 rf	CM 7
9.	W 117	643 × Minn. 13
10.	W 117 wx	W 117
11.	Mv L17	W 117 × B 37
12.	B 37	ISSS
13.	B 37 wx	B 37
14.	Mv L20	(W117 × B 37) × (B 37 × WF 9)
15.	Mv L10	Pool 51 (Iodent/Lancaster)
16.	Mv L8	Pool 51 (Iodent/Lancaster)
17.	Mv L9	Pool 1 (Iodent)
18.	Mv L12	Pool 52 (Iodent/Lancaster)
19.	Mv L11	Pool 1 (Iodent)
20.	Mo 17	187-2 × C 103
21.	Mo 17 wx	Mo 17
22.	Mv L4	(H 99 × Mo 17)H 99
23.	Mv L6	(Mo 17 × W 117)Mo 17
24.	Mv L7	(Mo 17 × W 117)Mo 17
25.	H 108	(Mo 17 × H 99)Mo 17
26.	Mv L19	Mo 17
27.	F 564	F7 × F 64
28.	F 7	O. P. Lacaune

Genetic analyses: Genomic DNA was isolated from seedlings of maize inbred lines in the 2-leaf stage by extracting the sap from fresh young leaves in a Sap Extractor (Ravenel Sci.) using 750 μl extraction buffer containing Tris, EDTA, o-phenantrolin, NaCl, CTAB and 2-mercaptoethanol (Dweikat et al.,1994; Gyulai et al., 2000). The following steps were included:

Genomic DNA extraction: Genomic DNA was isolated from the sap samples in extracting buffer as follows: (1) Incubation: 1 h, 65°C; (2) Precipitation of proteins with 500 μl of a 24:1 mixture of chloroform and isoamyl alcohol after cooling the samples to room temperature; (3) Incubation: 1 h, –20°C; (4) Centrifugation: –4°C, 5 min, 10,000 rpm; (5) DNA precipitation with 800 μl (2/3 vol) isopropanol from the upper of the three phases, which contained the plant DNA, followed by incubation for 1 h at room temperature; (6) Centrifugation: 4°C, 5 min, 10,000 rpm; (7) Rinsing of the DNA precipitate with 70% ethanol (500 μl) after decanting the isopropanol, followed by centrifugation (–4°C, 5 min, 10,000 rpm); (8) Drying of the DNA precipitate for 30 min at room temperature after decanting the alcohol; (9) Dissolution of DNA in 300 μl TE buffer (Dweikat et al., 1994; Gyulai et al., 2000).

Removal of RNA by RNase treatment: RNA was removed from the genomic DNA samples by treatment with 5 μl RNase-A (1 h, 37°C), after which the DNA samples were re-precipitated for 1 h at room temperature with a mixture of 30 μl 1/10 vol. 8 M ammonium acetate and 600 μl 2 vol. ethanol (70%, –20°C). After centrifugation (–4°C, 5 min, 10,000 rpm) the DNA precipitate was dissolved in 300 μl TE buffer. The quantity of DNA was measured using an OD260 UV spectrometer and the final DNA concentration of 5 ng/10 μl was adjusted in a dilution series on 0.8% agarose gel electrophoresis.

PCR reactions: The components were added in the following concentrations: 10×Taq buffer with 15 mM $MgCl_2$: 2.5 μl; $MgCl_2$ (25 mM): 1 μl, 1 mM; dNTPmix, dATP:dGTP:dCTP:dTTP = 1:1:1:1 (40 mM): 0.3 μl, each 7.5 nM; primer (80–100 nM): 3 μl, 0.Mv L2 nM; RedTaq (Sigma): 1 μl / 1 U; iH_2O (9.4 μl); and sample DNA: 3 μl, 1–3 ng.

Oligonucleotide primers: The maize inbred lines were tested with 20 RAPD and 20 SSR primers. The random polymorphisms were analysed using a 10 bp OP/AB set (Operon Sci., USA), while the genetic polymorphisms occurring in the repetitive DNA sequences were analysed using 16–18 bp SSR primers ($[GACA]_4$; $AC[GACA]_4$; $[GACA]_4AC$; $CA[GACA]_4$; $[GACA]_4CA$; $[ACTG]_4$) (Weining and Langridge, 1991; Gupta et al., 1994; Zietkiewicz et al., 1994).

Gel electrophoresis: The PCR reaction products were separated by electrophoresis (2 h at 80 V for RAPD and 40 V for SSR) on a 1.6% (RAPD) or 4% (SSR) agarose gel stained with 0.5 mg/ml ethidium bromide. The characteristic PCR reactions were repeated at least three times. Only the main PCR fragments appearing in all the repeated reactions were taken into consideration during the evaluation.

Statistical evaluation of the results: The data were evaluated using the SPSS (Norusis, 1993) cluster analysis programme on the basis of standardised morphological data, and a presence-absence data matrix for the alleles of PCR fragments and enzyme loci exhibiting polymorphism (Gyulai et al., 2000; Nagy et al., 2000).

Results and discussion

The isoenzyme analysis was successful for all the enzyme loci. Polymorphisms were determined by comparing pairs of lines.

Of the 15 isoenzyme loci examined, ten exhibited polymorphism; 23 of the possible 31 alleles at these loci were found in the tested lines. Four loci for the MDH enzyme and one for ADH exhibited no polymorphism, so these were disregarded when determining degrees of relationship. In the RAPD analysis the results given by five of the 20 primers tested could not be evaluated unambiguously, while three were monomorphic. This left 12 primers, with a total of 93 fragments, 54 of which exhibited reliable polymorphic patterns in all the replications (Fig. 1). In the SSR studies, the PCR patterns of two of the 20 primer pairs could not be evaluated and three were monomorphic. Although a further four primer pairs exhibited polymorphism, only differences amounting to a few base pairs could be clearly distinguished between the fragments, so the data matrix was prepared on the basis of 71 polymorphic fragments arising from 11 primer pairs (Fig. 2).

Judging from the isoenzyme patterns, the 28 lines formed a total of 16 gel electrophoresis groups, indicating that some lines could not be distinguished on the basis of enzyme patterns. With the help of RAPD and SSR-PCR analyses, polymorphism could be detected for all the lines. For the two lines which exhibited the greatest similarity, differences were observed in six fragments after RAPD analysis and in two fragments with SSR analysis.

An analysis of genetic relationships revealed that different methods gave different pictures of the genetic links between the lines. Groupings based on morphological descriptions, isoenzyme patterns (Fig. 3) and RAPD analysis exhibited only approximate agreement with the genetic relationships expected on

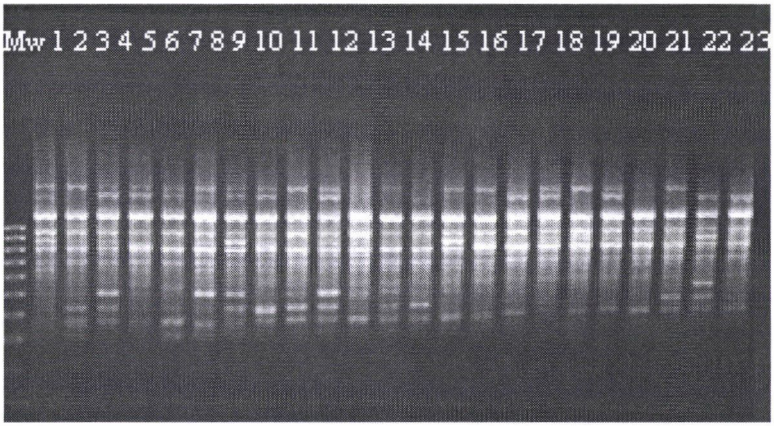

Fig. 1. Molecular analysis of maize inbred lines using the *OP/AB-20* RAPD primer (CTT CTC GGA C)

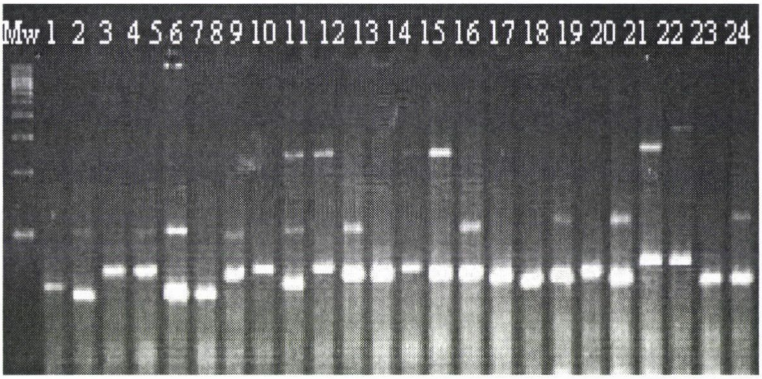

Fig. 2. Molecular analysis of maize inbred lines applying the *o2* (opaque endospermium protein 2 gene) gene-specific microsatellite primer pair (r: ATG GAG CAC GTC ATC TCA ATG G, f: AGC AGC AGC AAC GTC TAT GAC ACT)

the basis of pedigree. The SSR patterns reflected the real relationships to the least extent (Fig. 4), but this was due to the low number of primer pairs, not to the inefficiency of the method. As the SSR markers are to be found in the hypervariable regions of the chromosomes, a far larger number of primer pairs are required than in the analysis of isoenzymes, which act as structural genes. Evaluation using various combinations of markers indicated that the fewer the number of lines belonging to a genetic group, the fewer marker systems needed to be analysed together to obtain an accurate picture of degrees of relationship. This means that when the number of lines exceeds the limits of analysis for the given marker, the best solution is not always to expand the number of primers or enzymes used for marker analysis; the correct combination of various genetic markers may give more exact results, suitable for evaluation. This is also important from the point of view of expense, since costly analyses such as SSR can be combined with cheaper isoenzyme analysis. The genetic background was reflected most accurately by a dendrogram produced by processing the results obtained with three genetic methods, combined with morphological data (Fig. 5). The various groups could be clearly distinguished from each other, while within each group, with the exception of line Mv L2, all the maize inbred lines were placed according to their origin. This exception can probably be explained by the fact that the ISSS group contained more lines than the resolution of the method could cope with.

It thus appears that if a system is to be developed which allows the genetic background of genotypes of unknown origin to be revealed, thus facilitating the prediction of heterosis, it will need to combine both dominant (RAPD, AFLP, etc.) and codominant (SSR, RFLP, isoenzyme, etc.) markers, applied together with the heterosis test, morphological descriptions and pedigree analysis.

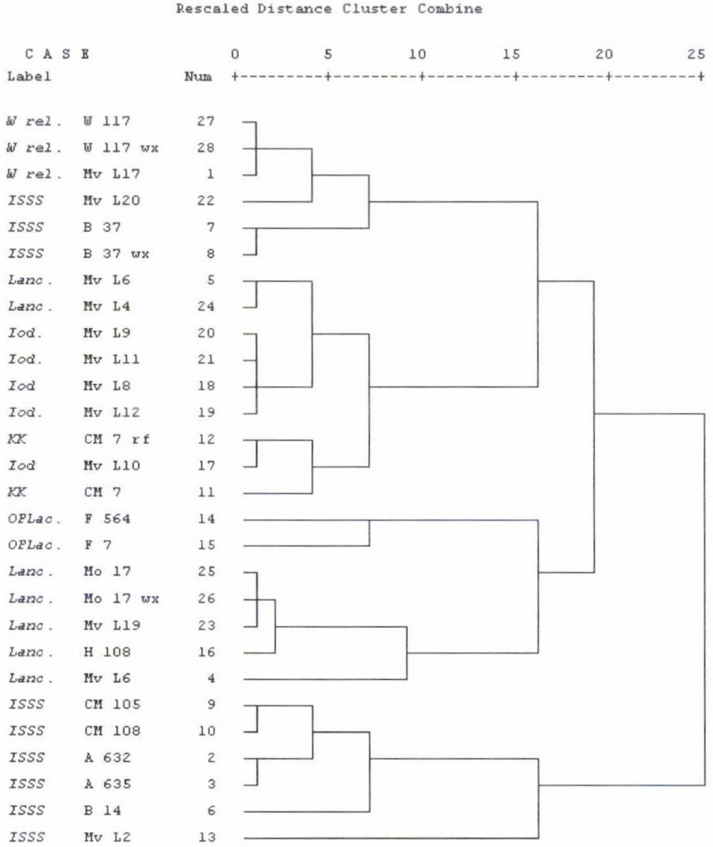

Fig. 3. Related groups on the basis of isoenzyme patterns
(Related groups: *Iod.*: Iodent; *ISSS*: Iowa Stiff Stalk Synthetic; *Lanc.*: Lancaster; *OPLac.*: OP Lacaune; *KK*: Early Canadian; *W rel.*: lines related to W 117)

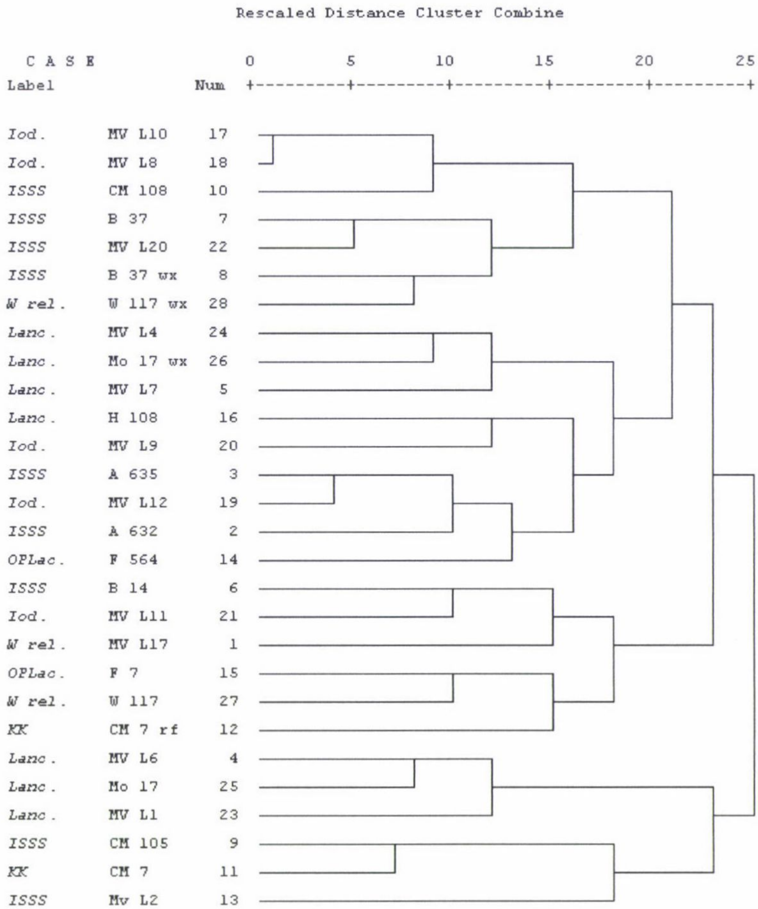

Fig. 4. Related groups on the basis of SSR markers (Related groups: *Iod.*: Iodent; *ISSS*: Iowa Stiff Stalk Synthetic; *Lanc.*: Lancaster; *OPLac.*: OP Lacaune; *KK*: Early Canadian; *W rel.*: lines related to W 117)

In summary it can be said that molecular marker analyses are now an important part of breeding. They can be applied routinely to test for polymorphism, thus playing an indispensible role in checking the homogeneity of lines and hybrids and the genetic stability and purity of breeding stocks, as well as forming a basis for the protection of intellectual property rights.

The grouping of maize varieties according to their genetic background is of outstanding importance in breeding, since a sufficient genetic distance between the parental lines is a fundamental criterion for heterosis breeding. Maize lines can be put into groups which give an accurate and reliable reflection of their genetic background by combining various marker systems, supplemented with morphological data. In this way genetic markers can be of service to breeders in the planning of crossing programmes.

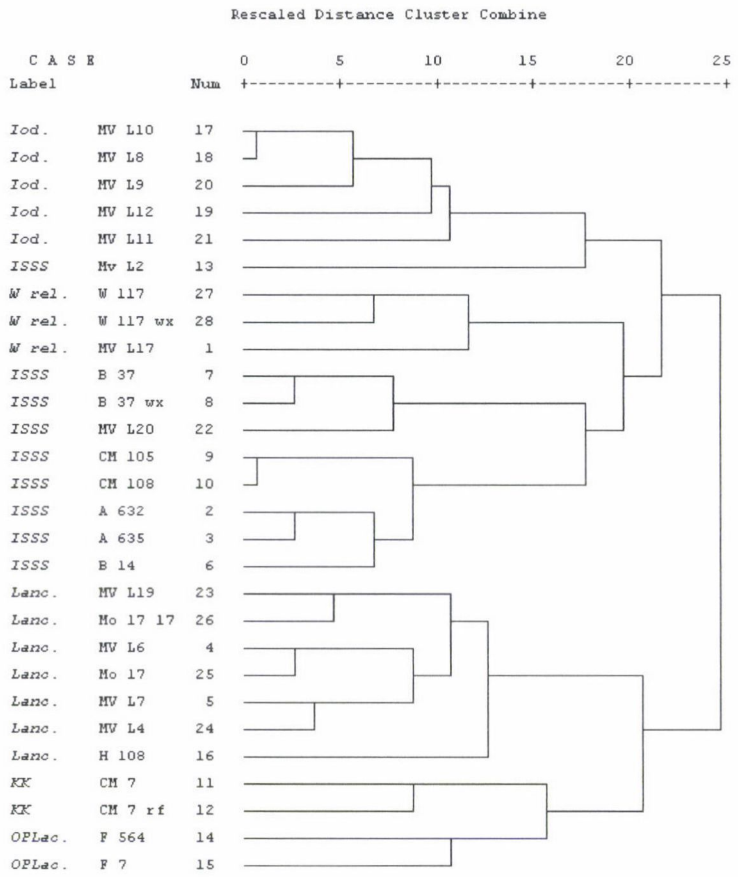

Fig. 5. Comprehensive cluster analysis of the related groups on the basis of genetic markers and morphological traits (Related groups: *Iod.*: Iodent; *ISSS*: Iowa Stiff Stalk Synthetic; *Lanc.*: Lancaster; *OPLac.*: OP Lacaune; *KK*: Early Canadian; *W rel.*: lines related to W 117)

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GENOTYPIC VARIATION FOR POTASSIUM ACCUMULATION AND UTILIZATION EFFICIENCY IN BARLEY UNDER RAINFED POTASSIUM STRESS CONDITIONS

Y. S. SHIVAY[1], J. H. CHEN and S. R. DING

DEPARTMENT OF AGRONOMY, ZHEJIANG AGRICULTURAL UNIVERSITY, HANGZHOU, CHINA
[1]PRESENT ADDRESS: DIVISION OF AGRONOMY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

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A field experiment was carried out to study the effect of K nutrition and genotypic variation on the dry matter (DM) accumulation, and the K concentration, accumulation, uptake and utilization efficiency in barley (*Hordeum vulgare* L.). Successive increases in potassium nutrition had a significant effect on the dry matter and K accumulation either in the total or in various plant parts of barley at the tillering, stem elongation, heading and maturity growth stages. K nutrition also led to significantly higher grain yield with each unit K application than without K application. The yield increase due to K application was mainly due to the improvement in spike development from tillers. Dry matter and K accumulation in various plant parts varied significantly between genotypes at the main growth stages. Among the various plant parts, the stem contained the highest K concentration, had the highest K accumulation at maturity and changed considerably with the K level, while other plant parts remained relatively unchanged. Among the eleven genotypes, genotype 98-6 had the highest grain yield and the K use efficiency of this genotype was 10.4 kg grain per kg K applied. It could thus be used as a breeding line to breed barley varieties for higher productivity under rainfed conditions with low available soil potassium.

Key words: barley, potassium, nutrition, genotype, dry matter, K accumulation, K use efficiency

Introduction

The development of crop cultivars which are more efficient in nutrient use or better adapted to nutritionally marginal environments requires effective and reliable techniques for the evaluation of nutrient use efficiency (NUE). Such techniques should allow a meaningful discrimination between efficient and inefficient genotypes to enable breeders to select effectively. For crops whose value product is reproductive, nutrient use efficiency can be evaluated on the basis of vegetative growth or economic product (Woodend and Glass, 1993). In most instances measures based on vegetative growth have been used to characterize different genera, species or genotypes for NUE. They include the presence or absence of deficiency symptoms (Diers and Fehr, 1989; Jessen et al., 1986; Wann and Hill, 1973), absolute growth at a limiting nutrient level (Chisholm and Blair, 1988; Shea et al., 1967; Schettini et al., 1987), relative

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growth rate obtained by comparing growth at limiting and adequate nutrient levels (Coltman et al., 1985; Gerloff, 1987), efficiency ratio or amount of biomass produced per unit of nutrient present in the tissues (Coltman et al., 1985; Makmur, 1977; O'Sullivan et al., 1974; Schettini et al., 1987; Woodend, 1986; Woodend et al., 1987; Woodend and Glass, 1993) and utilization index (Siddiqi and Glass, 1981; Woodend, 1986; Woodend and Glass, 1993). The utilization index, which is defined as biomass per unit of tissue nutrient concentration, was proposed by Siddiqi and Glass (1981) as an improved measure which, unlike the efficiency ratio, takes into consideration differences in the amount of biomass produced. While such measures are relatively easy to obtain, they may not reflect, or be correlated with economic nutrient use efficiency.

Although reports on genetic variation for NUE are legion, it has been assessed on the basis of field performance or economic yield only in limited studies. Woodend et al. (1987) and Woodend and Glass (1993) studied the uptake and utilization of potassium in wheat under potassium stress conditions and found some variation between varieties and genotype environment interactions. In view of the increasing interest in breeding for improved NUE, there is a need to determine whether the vegetative measures of NUE are positively correlated with those based on economic yield. It is important to identify genotypes with higher NUE and understand the mechanism of differences in the response to fertilizer application and soil nutrients between genotypes, in order to breed for high nutrient efficiency. Barley is the third largest crop in planting area after rice and wheat in China, and it requires a relatively higher K nutrient level for normal growth and development in comparison with other cereal crops. In China about 70% of soils are deficient in K for barley production, and now most K fertilizers are being imported. Hence, it is important to breed barley varieties with high K use efficiency. The experiment was conducted to study the genotypic variation in potassium utilization efficiency among barley genotypes with the following objectives: (1) to study the dry matter and K accumulation at increased levels of K nutrition in different barley genotypes; (2) to study differences in the uptake and distribution of potassium in barley genotypes; (3) to identify genotypes which are more efficient in potassium uptake and utilization efficiency for further use in breeding improvement programmes.

Materials and methods

A field experiment was carried out during the winter season of 1998–99 at the experimental farm of Zhejiang Agricultural University, Hangzhou, China, to study the effect of potassium nutrition and genotypes on the partitioning of dry matter and potassium accumulation in different plant parts of barley at the main crop growth stages and the K use efficiency at harvest under rainfed, potassium stress conditions. Eleven barley genotypes with different morphology and yield components were planted at four levels of potassium (0, 62, 124 and 186 kg K per ha as potassium chloride) in a split-plot design with three replications. The potassium levels were applied in the main plots, while the barley genotypes formed the sub-plots. Each genotype was planted in 6 rows 1.8 m in length, with 0.24 m between rows. The available potassium content (extracted with 1 *N* NH_4OAc and determined by flame photometry) was 35 mg per kg soil and the hydrolytic nitrogen content (determined by the microdiffusion method with alkali, 1.0 *N* NaOH) was 0.46 mg per 100 g of soil in the experimental soil. Nitrogen and phosphorus fertilizers were applied @ 250 and 80 kg per ha, respectively, in the form of urea and superphosphate. Throughout the growing season of barley no irrigation was applied to the growing crop as there was well distributed rainfall.

Measurements of dry matter and potassium accumulation were recorded at the main growth stages, namely tillering, stem elongation, heading and maturity. At every growth stage twenty plant samples were chosen randomly from each plot with identified sampling rows and thoroughly washed, first with tap water and then with deionized water. All plant samples were separated into roots, leaves and sheath at the tillering and stem elongation stages and into roots, leaves, stem and ear, etc. at heading and maturity. The samples were oven dried at 80–85°C for 24 hours before recording dry weight and then ground in a cyclone mill. Before the determination of K concentration by flame photometry, the samples were moist-ashed with H_2SO_4-H_2O_2. Potassium accumulation in different plant parts was recorded with their respective K concentrations and dry matter (DM) weight. At maturity, 20 plants from each plot were also harvested randomly for the determination of dry matter weight, K concentration and yield characters. The following parameters were estimated according to the dry matter and K concentration of various organs: (1) K accumulation: dry matter weight × K concentration; (2) K use efficiency (kg grain per kg K applied):

$$\frac{\text{Grain yield in fertilized plot} - \text{grain yield in control plot}}{\text{Fertilizer K applied}}$$

Statistical analysis

All the data recorded during experimentation were subjected to computer analysis in split-plot design software according to Duncan's multiple range test significant at 95 and 99% probability.

Results and discussion

Dry matter accumulation – Tillering

There was a highly significant effect of K nutrition on dry matter accumulation in the leaf, sheath, and total plant (mg plant^{-1}). Successive increases in K levels led to a significant increase in dry matter accumulation, and the maximum dry matter accumulation was recorded at the highest level of K nutrition (Table 1). This might be due to the good growth of the plants at a higher level of potassium nutrition. There were also significant differences in the responses of the eleven tested barley genotypes to K nutrition with regard to dry matter accumulation. Genotype 97-7 had the highest dry matter accumulation (391.0 mg plant^{-1}), followed by genotype 98-40 (376.9 mg plant^{-1}), while the lowest dry matter accumulation was recorded for genotype 98-30 (131.4 mg plant^{-1}). This was because of differences in the leaf number, leaf area and tillers/culm between the different genotypes tested. At the tillering stage more dry matter accumulation was recorded in the leaves compared to the sheath, irrespective of the K nutrition and genotypes.

Table 1
Effect of K nutrition and genotypes on dry matter accumulation in barley plant parts at the tillering and stem elongation growth stages

Treatment	Tillering (mg plant^{-1})			Stem elongation (g plant^{-1})		
	Leaf	Sheath	Total	Leaf	Sheath	Total
K levels (kg ha^{-1})						
0	99.2dD	71.1dD	170.3dD	0.55dD	0.51dD	1.06dD
62	120.0cC	90.9cC	210.9cC	0.66cC	0.61cC	1.27cC
124	139.7bB	121.2bB	260.9bB	0.75bB	0.71bB	1.46bB
186	156.3aA	147.1aA	306.4aA	0.84aA	0.81aA	1.65aA
Genotypes						
98-6	90.5fD	52.0hH	142.5gF	0.59eE	0.51fF	1.10deD
98-7	91.0fD	58.2gG	149.2fF	0.53fF	0.51fF	1.04cD
98-13	78.5gE	55.0ghGH	133.5hG	0.56efEF	0.50fF	1.06deD
98-30	75.2gE	56.2ghGH	131.4hG	0.45gG	0.40gG	0.85fE
98-35	98.2eD	91.7eE	189.9eE	0.56efEF	0.60eE	1.16dD
98-40	187.4bAB	189.5bB	376.9aA	0.94aA	0.83bB	1.77bB
97-7	195.8aA	195.aA	391.0aA	0.67dD	0.72cC	1.39cC
96-6	179.0cB	171.2cC	350.2bB	0.91aA	0.85bB	1.76bB
96-30	138.0dC	83.0fF	221.0dD	0.84bB	0.63deDE	1.47cC
96-34	138.7dC	79.5fF	218.2dD	0.75cC	0.67dCD	1.42cC
Nongda 3	144.2dC	151.5dD	295.7cC	0.92aA	1.17aA	2.09aA

Different small and capital letters in a column indicate significant differences at the 95% probability level and highly significant differences at the 99% probability level, respectively, according to Duncan's multiple range test

Stem elongation

The dry matter accumulation recorded at the stem elongation growth stage is given in Table 1. There were obvious differences in dry matter accumulation in the leaf and sheath with successive increases in K nutrition. The maximum dry matter accumulation was recorded at the highest level of K nutrition and the least in the control. This signified the importance of K nutrition for barley crops under rainfed, potassium stress conditions. There were also highly significant differences between the tested genotypes with regard to dry matter accumulation in the leaf, sheath and whole plant. At stem elongation the highest dry matter accumulation was recorded for genotype Nongda-3 (2.09 g plant^{-1}), followed by genotypes 98-40 and 96-6, while the lowest dry matter accumulation was recorded for genotype 98-30. This was attributed to differences in the morphology and physiology of the genotypes. There was equal contribution to the total dry matter accumulation through the leaf and sheath at the stem elongation stage, irrespective of the K nutrition and genotypes.

Heading

At the heading stage the barley plants were separated into three parts, namely leaf, stem and ear, etc. Dry matter accumulation in these three parts of the plant differed significantly due to K nutrition. The maximum dry matter accumulation was recorded at the highest level of K nutrition and was 36.4, 20.4 and 8.7% higher than in the control, 62 and 124 kg K ha^{-1} treatments, respectively (Table 2). This might be due to the improvement in plant growth at higher levels of K nutrition, as reported by Haeder and Beringer (1981). There was also highly significant variation between the tested barley genotypes with regard to dry matter accumulation in the leaf, stem, ear and plant. The highest dry matter accumulation was recorded for genotype 98-7 (3.67 g $plant^{-1}$), followed by genotype 96-34 (3.61 g $plant^{-1}$), with the lowest in genotype 98-30 (2.46 g $plant^{-1}$). This significant variation in dry matter accumulation in different genotypes might be due to the variation in tillering behaviour, leaf canopy and leaf area, which led to variations in dry matter accumulation, irrespective of the plant parts. At the heading stage the maximum contribution to the total dry matter accumulation was through the stem, followed by the leaf and ear, irrespective of the treatments.

Maturity

K nutrition had a highly significant effect on dry matter accumulation in the leaf, stem, ear and whole plant. The maximum dry matter accumulation (2.26 g $culm^{-1}$) was recorded at the highest level of K nutrition and was 26.9, 13.5 and 6.1% higher than in the 0, 62 and 124 kg K ha^{-1} treatments, respectively. This might be due to the increased accumulation of photosynthates in plants with a higher level of K nutrition (Zhang et al., 1999). There was also significant variation between the tested barley genotypes with regard to dry matter accumulation in the leaf, stem, ear and whole plant. The highest dry matter accumulation was recorded for genotype 96-6 (2.36 g $culm^{-1}$), which was statistically significantly superior to the rest of the genotypes (Table 2). Five other genotypes, namely 98-6, 96-30, 96-34, 98-35 and 98-30, remained statistically at par with each other in respect of DM accumulation. This might be due to similarity in the morphological and physiological parameters of the genotypes. At maturity, the maximum contribution to the total dry matter accumulation was through the ear, followed by the stem and leaf. This was because maximum photosynthates were accumulated in the sink (ear) from the source (leaf), irrespective of the K nutrition and genotypes.

Root-shoot dry matter accumulation

Root-shoot dry matter accumulation was recorded at the three main growth stages, namely tillering, stem elongation and heading (Table 3). There was a highly significant effect of K nutrition on root and shoot DM accumulation. Successive increases in K nutrition led to a significant increase in

Table 2

Effect of K nutrition and genotypes on dry matter accumulation in barley plant parts at the heading and maturity stages

Treatment	Heading				Maturity			
	Leaf (g plant^{-1})	Stem (g plant^{-1})	Ear (g plant^{-1})	Total (g plant^{-1})	Leaf (g plant^{-1})	Stem (g plant^{-1})	Ear (g plant^{-1})	Total (g plant^{-1})
K levels (kg ha^{-1})								
0	0.42d D	1.84d D	0.38c C	2.64d D	0.12d C	0.72d C	0.95d C	1.78d D
62	0.48c C	2.09c C	0.42c BC	2.99c C	0.13c BC	0.77c B	1.08c B	1.99c C
124	0.55b B	2.30b B	0.46b AB	3.31b B	0.14b B	0.80b B	1.18b AB	2.13b B
186	0.60a A	2.49a A	0.51a A	3.60a A	0.16a A	0.84a A	1.26a A	2.26a A
Genotypes								
98-6	0.58a A	2.42b B	0.51a AB	3.52bc AB	0.15cd BCD	0.72f EF	1.24a A	2.11b B
98-7	0.52cd BC	2.66a A	0.49ab ABC	3.67a A	0.11gh G	0.62g G	1.02c C	1.75d C
98-13	0.51de BC	2.15c C	0.45de DE	3.10e D	0.14de CDE	0.69f F	1.13abc ABC	1.97c B
98-30	0.35g E	1.73e E	0.38f F	2.46h G	0.10h G	0.76e DE	1.17ab ABC	2.03bc B
98-35	0.48e C	2.37b B	0.47bcd CDE	3.31d C	0.12fgh EFG	0.68de D	1.19a AB	2.09b B
98-40	0.58ab A	2.35b B	0.52a A	3.45c B	0.12fg EFG	0.79de CD	1.06bc BC	1.96c B
97-7	0.43f D	1.80d DE	0.34g F	2.65g F	0.13ef DEF	0.82cd BCD	1.02c C	1.98c B
96-6	0.57ab A	1.89d D	0.44e E	2.90f E	0.17a A	0.94a A	1.24a A	2.36a A
96-30	0.52cd BC	1.82de DE	0.35fg F	2.70g F	0.17ab AB	0.79de CD	1.15ab ABC	2.11b B
96-34	0.54bc AB	2.59a A	0.48bc BCD	3.61ab A	0.16bc ABC	0.87b B	1.07bc BC	2.10b B
Nongda 3	0.57ab A	2.19c C	0.45 cde DE	3.21de CD	0.11gh FG	0.84bc BC	1.02c C	1.97c B

Different small and capital letters in a column indicate significant differences at the 95% probability level and highly significant differences at the 99% probability level, respectively, according to Duncan's multiple range test

Table 3

Root and shoot dry matter accumulation in barley at the main growth stages as influenced by K nutrition and genotypes

Treatment	Tillering			Stem elongation			Heading		
	Root (mg plant^{-1})	Shoot (mg plant^{-1})	Root:Shoot	Root (g plant^{-1})	Shoot (g plant^{-1})	Root:Shoot	Root (g plant^{-1})	Shoot (g plant^{-1})	Root:Shoot
K levels (kg ha^{-1})									
0	30.4dD	170.3dD	0.18	0.10dD	1.06dD	0.09	0.28dD	2.64dD	0.10
62	36.6cC	210.9cC	0.17	0.11cC	1.27cC	0.08	0.31cC	2.99cC	0.10
124	43.3bB	260.9bB	0.16	0.13bB	1.46bB	0.09	0.34bB	3.31bB	0.10
186	50.5aA	306.4aA	0.24	0.15aA	1.65aA	0.09	0.37aA	3.60aA	0.10
Genotypes									
98-6	32.5gF	142.5gF	0.23	0.16aAB	1.10deD	0.14	0.45aA	3.52bcAB	0.13
98-7	38.2deCDE	149.2fF	0.25	0.16aA	1.04eD	0.15	0.42bB	3.67aA	0.11
98-13	38.7cdeCDE	133.5hG	0.29	0.12cdDE	1.06deD	0.11	0.38cB	3.10eD	0.12
98-30	36.0efDEF	131.4hG	0.27	0.09fF	0.85fE	0.10	0.23gF	2.46hG	0.09
98-35	34.7fgEF	189.9eE	0.18	0.12cdeDE	1.16dD	0.10	0.34dC	3.31dC	0.10
98-40	39.7cdCD	376.9aA	0.10	0.14bBC	1.77bB	0.08	0.27fE	3.45cB	0.08
97-7	42.0cBC	391.0aA	0.11	0.11deDE	1.39cC	0.08	0.25fEF	2.65gF	0.09
96-6	50.2aA	350.2bB	0.14	0.13bcCD	1.76bB	0.07	0.33deC	2.90fE	0.11
96-30	45.2bB	221.0dD	0.20	0.11deEF	1.47cC	0.07	0.28fDE	2.70gF	0.10
96-34	45.5bB	218.2dD	0.21	0.10efEF	1.42cC	0.07	0.32deCD	3.61abA	0.09
Nongda 3	39.5cdCD	295.7cC	0.13	0.13cCD	2.09aA	0.06	0.31eCD	3.21deCD	0.09

Different small and capital letters in a column indicate significant differences at the 95% probability level and highly significant differences at the 99% probability level, respectively, according to Duncan's multiple range test

DM accumulation, and the maximum DM accumulation was recorded at the highest level of K nutrition at all three crop growth stages (Table 3). This was attributed to the improvement in growth and development of the plants at higher levels of K nutrition. There was highly significant variation in the root-shoot DM accumulation between the tested barley genotypes at the tillering, stem elongation and heading stages. The root-shoot DM accumulation was inconsistent and varied among the genotypes at all three main growth stages. This might be due to variation in the morphology and physiology of the genotypes, which might also exhibit differences in their response to K nutrition at different growth stages. The highest root:shoot ratio was recorded at the tillering stage, irrespective of K nutrition and genotypes, compared to the stem elongation and heading stages. This might be due to the greater increase in shoot dry weight compared to root dry weight at the stem elongation and heading stages, respectively.

K accumulation at tillering and stem elongation

K accumulation in plants is a function of K concentration and dry matter (DM) weight. Table 4 shows the K accumulation in the leaf, sheath and whole plant at the tillering and stem elongation stages. There were significant differences in K accumulation with K nutrition. Each successive increase in K nutrition led to a significant increase in K accumulation and the maximum K accumulation (mg/plant) was recorded at the highest level of K nutrition at the tillering and stem elongation growth stages. This was because of the increase in K concentration and dry matter (DM) weight accumulation, which led to increased K accumulation in plant parts with increased K nutrition. There were also highly significant differences between the tested genotypes with regard to K accumulation (g plant^{-1}) at both stages of crop growth. Breeding line 98-40 and cultivar Nongda-3 had significantly higher K accumulation at the tillering and stem elongation growth stages, respectively, than other genotypes. This was because of the higher K concentration and DM accumulation in these genotypes than in the remaining genotypes. A higher contribution to K accumulation was recorded from the leaf compared to the sheath at the tillering and stem elongation growth stages, irrespective of K nutrition and genotypes. This was attributed to the larger quantity of photosynthates assimilated in the leaf during tillering and stem elongation compared to the sheath.

K accumulation at heading and maturity

K accumulation was recorded at heading and maturity in the leaf, stem, ear and whole plant (Table 5). There were highly significant variations in K accumulation in different plant parts and in the whole plant as the result of K nutrition. Each successive increase in K nutrition led to a significant increase in K accumulation in the leaf, sheath, ear and whole plant at both heading and maturity. This was due to increased K concentration and DM accumulation with

Table 4

K accumulation (mg $plant^{-1}$) in different plant parts of barley at the tillering and stem elongation growth stages as influenced by K nutrition and genotypes

Treatment	Tillering			Stem elongation		
	Leaf	Sheath	Total	Leaf	Sheath	Total
K levels*						
0	0.36dD	0.21dD	0.57dD	2.05dD	1.94dD	3.99dD
62	0.47cC	0.31cC	0.78cC	2.66cC	2.25cC	4.91cC
124	0.57bB	0.46bB	1.03bB	3.26bB	3.17bB	6.43bB
186	0.69aA	0.62aA	1.31aA	3.87aA	3.86aA	7.73aA
Genotypes						
98-6	0.35gFG	0.16ghF	0.51ghGH	2.26fF	2.08gG	4.34efDE
98-7	0.36fgF	0.19fgF	0.55gG	2.08gG	1.92hH	4.00fE
98-13	0.32hGH	0.19fF	0.51ghGH	2.26fF	2.00ghGH	4.26fDE
98-30	0.31hH	0.18fghF	0.49hH	1.77hH	1.64iI	3.41gF
98-35	0.38fF	0.31dDE	0.69fF	2.27fF	2.40fF	4.67eD
98-40	0.80aA	0.72aA	1.52aA	3.85cC	3.60cC	7.45bB
97-7	0.78aA	0.74aA	1.52aA	2.81eE	2.86eE	5.67dC
96-6	0.75bB	0.67bB	1.42bB	4.09aA	4.37bB	8.46aA
96-30	0.61cC	0.33dD	0.94dD	3.94bcBC	3.36dD	7.30bB
96-34	0.53eE	0.29eE	0.82eE	3.25dD	2.85eE	6.10cC
Nongda 3	0.57dD	0.60cC	1.17cC	3.99bAB	4.60aA	8.59aA

*(kg K ha^{-1}); For legends see Table 1

Table 5

K accumulation in different plant parts of barley at the heading and maturity stages as influenced by K nutrition and genotypes

Treatment	K accumulation (mg $plant^{-1}$)				K accumulation (mg $culm^{-1}$)			
	Heading				Maturity			
	Leaf	Stem	Ear	Total	Leaf	Stem	Ear	Total
K levels*								
0	1.16dD	3.57dD	0.39dD	5.12dD	0.06dD	1.67dD	0.52dD	2.25dD
62	1.50cC	4.90cC	0.48cC	6.88cC	0.08cC	2.04cC	0.67cC	2.79cC
124	1.82bB	6.05bB	0.54bB	8.41bB	0.11bB	2.43bB	0.80bB	3.34bB
186	2.13aA	7.09aA	0.65aA	9.87aA	0.15aA	2.77aA	0.93aA	3.85aA
Genotypes								
98-6	1.62deCD	4.77deE	0.57bB	6.96cD	0.06fF	1.95fD	0.68cdeC	2.69gF
98-7	1.31gF	4.97dE	0.53cdBC	6.81cC	0.03iF	1.46gE	0.58fD	2.07hG
98-13	1.46fF	4.55eE	0.54bcdBC	6.55cD	0.05ghE	2.05fD	0.71cdC	2.81fF
98-30	1.08hG	3.88fF	0.42eD	5.38dE	0.05hEF	2.04fD	0.70cdeC	2.79fgF
98-35	1.54efDE	4.97dE	0.51dC	7.02cD	0.06fgE	1.99fD	0.71cC	2.76fgF
98-40	1.71dC	5.81cD	0.66aA	8.18bC	0.12deCD	2.22deC	0.80bB	3.14dD
97-7	1.50efDE	4.76deE	0.40eD	6.66cD	0.12cdCD	2.31dC	0.66eC	3.09dD
96-6	2.08aA	6.33bBC	0.53cdBC	8.94aAB	0.16bB	3.11aA	0.85aA	4.12aA
96-30	2.06abA	6.58abAB	0.43eD	9.07aA	0.21aA	2.64bB	0.86aA	3.71bB
96-34	1.88cB	6.84aA	0.56bcBC	9.28aA	0.13cC	2.54cB	0.81bAB	3.48cC
Nongda 3	1.96bcAB	5.95cCD	0.51dC	8.42bBC	0.11eD	2.18eC	0.67deC	2.96eE

*(kg K ha^{-1}); For legends see Table 1

increased levels of K nutrition, as also reported by other authors (Woodend and Glass, 1993; Zhang et al., 1999). Genotypes showed large, inconsistent and highly significant differences in K accumulation either in the whole plant or in various plant parts. The total K accumulation of genotypes 96-30 and 96-6 was the highest at heading and maturity, respectively, and these values were significantly higher than for other genotypes. At heading and maturity most of the K was located in the stem and least in the ear. Because of the higher K concentration and larger DM, the stem had greater K accumulation, consisting of more than 70% of the total K accumulation in the plant. Therefore, it is important that barley straw is returned to the field as crop residue/manure in order to improve the K status of the soil for sustainable agriculture. This study showed genetic variations in K nutrient uptake between breeding lines. Moreover, K levels had an obvious influence on some of the vegetative variables related to nutrient efficiency. Similar studies on genotypic differences in nutrient uptake were studied by many workers in different crops (Zhang et al., 1999).

Grain and straw yields

There were highly significant effects of K nutrition on the grain and straw yields of barley with successive increases in K nutrition level (Table 6). This was because K fertilizer had a positive effect on yield attributes such as spikes per plant and grains per spike, which led to a significant increase in the grain and straw yield. The maximum grain yield (6.32 t ha^{-1}) was recorded at the highest level of K nutrition and was 7.7, 19.9 and 35.9% higher than in the 124, 62 kg K ha^{-1} and control treatments, respectively. These findings are well supported by Mishra et al. (1982) and Singh et al. (1987). There were also significant variations in the response to K nutrition between four genotypes with regard to grain and straw yields. Among the eleven genotypes, genotype 96-6 recorded the highest grain yield (6.57 t ha^{-1}) followed by Nongda-3. Three of the eleven tested genotypes, namely 98-40, 98-35 and 97-7 were at par with regard to grain and straw yields (Table 6). Genotype 98-7 had the lowest grain and straw yields. Raikwar and Paradkar (1987) and Woodend and Glass (1993) also reported variation between varieties and genotype-environment interactions under conditions of K stress.

K use efficiency

The response of barley genotypes to applied K (K use efficiency) varied from 8.9–10.1 kg grain per kg of K with the application of 186 kg K ha^{-1} and 62 kg K ha^{-1}, respectively. In general, as the K application rate was increased, the K use efficiency decreased. There were highly significant variations among the genotypes with regard to K use efficiency. Genotype 98-35 had the highest K use efficiency (12.6 kg grain per kg K applied), followed by genotype 98-40 (11.2 kg grain per kg K applied), while the lowest K use efficiency was recorded for genotype 98-6 (6.0 kg grain per kg K applied). Patterson and Jensen (1983) also observed great differences in the potassium efficiency ratio between 11 barley cultivars. This was also due to variation in the yield of different genotypes with the same level of K nutrition. The K fertilizer lengthened the period of grain filling both by advancing anthesis and delaying senescence. The longer grain filling period, fed by more abundant assimilates, resulted in higher K use efficiency (Haeder and Beringer, 1981).

Table 6
Effect of K nutrition and genotypes on grain yield, straw yield, K accumulation and K use efficiency in barley

Treatment	Grain ($t\ ha^{-1}$)	Straw ($t\ ha^{-1}$)	K accumulation ($kg\ ha^{-1}$)			K use efficiency (kg grain kg^{-1} K)
			Grain	Straw	Total	
K levels ($kg\ ha^{-1}$);						
0	4.65dD	5.19dD	19.83dD	70.40dD	94.23dD	–
62	5.27cC	5.87cC	24.07cC	96.17cC	120.24cC	10.1aA
124	5.87bB	6.47bB	28.30bB	123.66bB	151.95bB	9.7aA
186	6.32aA	6.87aA	32.16aA	145.34aA	177.50aA	8.9bB
Genotypes						
98-6	4.93fDE	5.48eDE	22.53eEF	85.68dE	108.21gG	6.0fF
98-7	4.61gE	5.22fE	19.84fF	70.55gF	90.39hH	9.2cdCD
98-13	5.05fD	5.61eD	23.64deDE	94.07eD	117.71fF	8.3deDE
98-30	5.40eC	5.98dC	24.82cdeCDE	95.54eD	120.36efEF	10.3bBC
98-35	5.70cdC	6.28cC	25.37cdCDE	98.41eD	123.78eE	12.6aA
98-40	5.73cC	6.32cC	29.25abAB	119.97cC	149.23cC	11.2bB
97-7	5.69cdC	6.29cC	27.24bcBC	118.65cdC	145.89cCD	7.7eE
96-6	6.57aA	7.06aA	31.61aA	150.91aA	182.52aA	10.4bBC
96-30	5.60cdeC	6.17cdC	26.25cBCD	141.28bB	167.53bB	10.2bcBC
96-34	5.43deC	6.03dC	27.10bcBC	114.56dC	141.66dD	10.2bcBC
Nongda 3	6.10bB	6.67bB	29.31abAB	119.21cC	148.52cC	9.1dCD

For legends see Table 1

The results of this study showed that K nutrition had a highly significant effect on dry matter (DM) and K accumulation both in the whole plant and in various plant parts of barley at different growth stages, namely tillering, stem elongation, heading and maturity. The results also revealed that eleven barley genotypes significantly differed genetically in their dry matter (DM) and K accumulation, either in the whole plant or in various plant parts, as well as in their grain and straw yields and K use efficiency. Among the various genotypes, genotype 96-6 gave a significantly higher grain yield than the other genotypes tested, with a K use efficiency of 10.4 kg grain per kg K applied. Genotype 96-6 could thus be used in barley breeding improvement programmes under rainfed, K stress conditions to develop efficient nutrient use and higher productivity. The present research indicated that high nutrient uptake is not necessarily correlated with high dry matter production. According to Saric (1983), genotypes with a small concentration of certain mineral nutrients and a high photosynthesis rate are the most economical, since these genotypes require the least amount of mineral fertilizer to produce high yields. The present study also revealed that there are significant differences between barley genotypes in K concentration and accumulation in plant parts and it is possible to breed varieties with high biomass production and lower K concentration in the aboveground plant parts to sustain barley productivity under rainfed conditions with low available soil potassium.

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COMBINING ABILITY IN THE F_1 AND F_2 GENERATIONS OF A DIALLEL CROSS IN SIX-ROWED BARLEY (*HORDEUM VULGARE* L.)

Y. SHARMA, S. N. SHARMA, P. JOSHI and R. S. SAIN

ALL INDIA COORDINATED WHEAT AND BARLEY IMPROVEMENT PROJECT, RAJASTHAN AGRICULTURE UNIVERSITY, AGRICULTURAL RESEARCH STATION, DURGAPURA, RAJASTHAN, INDIA

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The F_1 and F_2 progenies of a ten-parent diallel cross (excluding reciprocals) were analysed for the combining ability of quantitative traits in six-rowed barley (*Hordeum vulgare* L.). Significant differences were indicated between the parents, F_1s and F_2s for all the characters studied. The GCA and SCA components of variance were significant for all the traits. Both additive and non-additive gene effects were involved in the genetic control of the characters; however, non-additive gene effects were observed to be predominant. Among the parents RD 2035, RD 2052, RD 2503 and BL 2 were the best general combiners for grain yield and average to high combiners for other important traits. The parents RD 2552 and RD 387 were the best general combiners for dwarfness. The best specific crosses for grain yield were RD 2503 × RD 2585, RD 2035 × RD 2052, RD 2035 × BL 2, RD 2052 × BL 2, RD 2508 × RD 2552, RD 2552 × RD 2585 and Rd 2052 × RD 2552 in both the F_1 and F_2 generations. These crosses were higher yielders and in most of the crosses one of the parents involved was a good combiner, indicating that such combinations can be expected to produce desirable transgressive segregants. All the best crosses for grain yield also showed average to high SCA effects for most of the yield components. Most of the specific crosses for grain yield involved high × average, average × average and average × poor general combiners. To ensure a further increase in grain yield, the combination of desirable yield components is advocated. The inclusion of F_1 hybrids showing high SCA, and having parents with good GCA, in multiple crosses, bi-parental mating or diallel selective mating could prove a worthwhile approach for further amelioration of grain yield in six-rowed barley.

Key words: six-rowed barley, combining ability, diallel cross, gene effects, yield components

Introduction

Barley (*Hordeum vulgare* L., 2n = 2x = 14) is an important cereal crop grown throughout the temperate and tropical regions of the world. It has been a very important crop in India since ancient times and many efforts have been made by scientists to improve its productivity. By virtue of its nature, the lower cost of cultivation, superior nutritional qualities and many other uses, barley is very promising in many less favoured and neglected agricultural areas, particularly in problematic soils like rainfed, dry land, saline-alkaline soil and flood-prone marginal/coastal areas. In India, it is grown on more than 728.000 hectares, with a production of more than 1.456 million tonnes and a productivity of 20.01 q/ha. Barley is used as human food either for breadmaking (usually mixed with bread wheat but also with other cereals or food legumes) or for

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traditional recipes. The major use of barley grains is in the production of malt, which is used in breweries to make beer, industrial alcohol, whisky, malt syrups, malted milk and vinegar. The spent malt after brewing is used as feed.

Barley cultivation in India is now becoming oriented towards industrial utilization. Although at present only 12–15% of the total produce is utilized for malting/brewing, it is projected that by 2020 the demand will be more than double. Keeping in view the increasing concern for the quality of raw material (barley) to be used by industries, the ultimate challenge is for breeders to breed varieties with high yield potential along with high malting quality and greater stability for industrial utilization. The improved six-rowed malting genotypes with early maturing and better tillering can further bridge the yield gap and help to meet the demand for quality grain for malting purposes.

Grain yield, a complex metric trait, is an ultimate product of the action and interaction of a number of component characters. For an effective breeding programme, aimed at the improvement of complex characters such as yield, a sound knowledge of the genetics of yield and its related characters is essential to make a right choice of selection procedures for the skilful management of breeding material. Furthermore, the presence of adequate genetic variability in the breeding material is essential for an effective breeding programme (Allard, 1960). Advances in the yield of this important species requires information on the combining ability of the parents available in the genetic material to be used in the hybridization programme and on the nature of the gene actions involved in the expression of quantitative traits of economic importance. The general and specific combining ability effects are very effective genetic parameters in deciding the next phase of a breeding programme. According to Arunachalam (1976) and Baker (1978), combining ability is a useful biometrical tool for use in plant breeding programmes. Diallel analysis also provides a unique opportunity to test a number of lines in all possible combinations. Thus, the main objective of the present studies was to identify the best combining parents and their crosses on the basis of general and specific combining ability for yield and its component traits for the further amelioration of grain yield in barley.

Materials and methods

Ten varieties of six-rowed barley (*Hordeum vulgare* L.), namely, RD 2035, RD 2052, RD 2503, RD 2508, RD 2552, RD 2585, RD 387, BL 2, ISBYT 4 and ISBYT 17, were crossed in all possible combinations excluding reciprocals. The 10 parents and their resulting 45 F_1s and 45 F_2s were grown in a randomized block design with three replications at Asalpur Research Farm of SKN College of Agriculture, Jobner, Jaipur, Rajasthan, India. Plots of parents and F_1s consisted of two rows each 2 m in length, while each plot of F_2 consisted of four rows with a spacing of 30 cm between rows and 10 cm between plants. Ten competitive plants from the parents and F_1s and 30 plants from the F_2 progenies were selected randomly for recording observations on ten characters, namely days to heading (75%), plant height (cm), tillers per plant, flag leaf area (cm^2), spike length (cm), number of spikelets per spike, number of grains per spike, 1000-grain weight (g), harvest index (%) and grain yield per plant (g).

The mean of each plot was used for statistical analysis. The data were first subjected to the usual analysis for a randomized block design for pooled environments as well as for individual environments (Panse and Sukhatme, 1967). The combining ability analyses were carried out following Method II, Model I of Griffing (1956).

Results and discussion

Analysis of variance indicated significant differences between the parents for all the characters. Similarly the differences between F_1 hybrids and F_2 progenies were found to be significant for all the traits except for spike length in F_2, indicating the presence of diversity in the material (Table 1). Significant differences between parents vs F_1s and parents vs F_2s were also found for all the traits, revealing the existence of heterosis and inbreeding depression. Significant differences between the genotypes for grain yield and related traits in different sets of material were also reported by Sethi et al. (1987), Kudla et al. (1988), Leistrumaite (1989) and Bhatnagar and Sharma (1995; 1998).

Analysis of variance for combining ability revealed that the variance due to general combining ability (GCA) and specific combining ability (SCA) was highly significant for all the traits studied in both the F_1 and F_2 generations, (Table 2). Thus, both kinds of gene effects were important in controlling the inheritance of all the characters studied. However, the GCA : SCA ratio tilted normally in favour of SCA for all the traits in both the F_1 and F_2 generations indicating the preponderance of non-additive gene effects in the genetic control of the traits. The present findings thus supported the reports of Zao et al. (1991), Guo and Xu (1994), Phogat et al. (1995), Madic (1996), El-Seidy (1997a; b) and Bouzerzour and Djakoune (1998), which clearly indicated that non-additive genetic variance was the main component of genetic variance for various economic traits in barley. However, the preponderance of additive effects was reported by Martinez (1984), Abdulamonov and Nigmatullin (1985), Kalashnik and Smyalovskaya (1986) and Yang and Lu (1991) and the role of both additive and non-additive effects was reported by Choo et al. (1988) and Bhatnagar and Sharma (1995; 1998) for grain yield and its component characters in barley.

Table 1

Analysis of variance showing mean squares for parents, F_1s and F_2s for different characters of barley

Characters	Replication	Genotype	Parents	F_1	F_2	P vs F_1	P vs F_2	Error
df	2	99	9	44	44	1	1	198
Days to heading	3.28	42.92**	38.87**	36.51**	45.58**	284.98**	218.73**	7.01
Plant height	12.02	208.50**	217.08**	215.60**	202.62**	283.93**	157.63**	14.72
Tillers per plant	0.31	1.80**	0.75*	1.75**	1.55**	22.27**	25.07**	0.35
Flag leaf area	0.44	1.70**	2.33**	1.74**	1.46**	5.31**	5.91**	0.35
Spike length	0.24	0.74**	1.04**	0.60**	0.53	14.35**	11.65**	0.38
No. of spikelets/spike	4.90	58.82**	32.82**	61.86**	54.41**	391.32**	350.96**	8.94
No. of grains/spike	0.54	58.42**	32.12**	59.51**	57.70**	329.45**	271.99**	6.07
1000-grain weight	1.78	27.23**	27.72**	27.78**	26.82**	39.21**	14.96**	1.83
Harvest index	1.59	6.17**	6.30**	5.37**	5.77**	58.17**	22.99**	1.75
Grain yield per plant	0.58	34.12**	13.49**	35.68**	29.96**	375.41**	227.29**	0.97

* and ** Significant at the 5 and 1 % levels, respectively.

Table 2
Analysis of variance for combining ability for different characters of barley

Characters	Source							
	GCA		SCA		Error		GCA/SCA	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
d.f.	9		45		108		–	
Days to heading	33.78**	33.79**	9.85**	12.31**	1.16	1.85	0.333	0.246
Plant height	183.91**	179.85**	50.06**	45.71**	4.39	4.54	0.289	0.328
Tillers per plant	1.32**	1.23**	0.52**	0.50**	0.08	0.09	0.160	0.165
Flag leaf area	1.90**	1.63**	0.38**	0.35**	0.07	0.08	0.464	0.468
Spike length	0.42**	0.38**	0.29**	0.25**	0.06	0.06	0.069	0.082
No. of spikelets/spike	41.02**	39.55**	17.04**	14.61**	2.89	3.07	0.141	0.180
No. of grains/spike	36.43**	41.99**	16.69**	14.57**	1.99	2.40	0.112	0.188
1000-grain weight	24.61*	23.51**	6.27**	6.00**	0.61	0.76	0.270	0.279
Harvest index	7.77**	4.22**	1.65**	1.64**	0.63	0.54	0.254	0.201
Grain yield/plant	29.60**	27.03**	9.39**	6.88**	0.30	0.34	0.185	0.257

* and ** Significant at the 5 and 1% levels, respectively.

The estimates of general combining ability (GCA) effects revealed that among the parents, RD 2035, RD 2052, RD 2503 and BL 2 were the best general combiners for grain yield and good to average combiners for most of the yield component characters (Table 3). However, the rest of the parents were poor combiners for grain yield and average to poor general combiners for most of the yield contributing traits. Parents RD 2552 and RD 387 were the best general combiners for dwarfness. Very similar trends of parents for general combining ability effects were observed in both the F_1 and F_2 generations. Parent RD 2035 was a good combiner for dwarfness, tillers per plant, flag leaf area and 1000-grain weight; RD 2052 was a good general combiner for early heading, dwarfness, tillers per plant, flag leaf area, spike length, number of spikelets per spike, 1000-grain weight and harvest index; parent RD 2503 was a good combiner for tillers per plant, flag leaf area, number of spikelets per spike, 1000-grain weight and harvest index (F_2); BL 2 was a good combiner for tillers per plant and flag leaf area. Apparently, therefore, there is still further scope for improving the combining ability for component traits, as none of the high combiners for grain yield was a high combiner or at least an average combiner for all the desirable traits. Parent RD 2508 was a high combiner only for dwarfness, spike length and 1000-grain weight, while parents RD 2585, ISBYT 4 and ISBYT 17 were found to be poor general combiners for grain yield and its contributing traits (Table 3).

High GCA effects are mostly due to additive gene effects or additive × additive interaction effects, as earlier reported by Griffing (1956). In view of this, breeders can utilize good general combiners in specific breeding programmes for the amelioration of grain yield in barley. It seems feasible, therefore, that the GCA rank for grain yield is related to the GCA for useful yield components. Breeders are therefore recommended to breed for superior combining ability for the component traits, with the ultimate objective of improving the overall GCA for grain yield in barley. The parents RD 2035, RD 2052, RD 2503 and BL 2 should be utilized extensively in the hybridization programme to accelerate the pace of genetic improvement for grain yield in six-rowed barley.

In order to synthesize a dynamic population with most of the favourable genes accumulated, the aforesaid parents, which are good general combiners for several characters, should be used in multiple crossing programmes. Apart from conventional breeding methods, which give slow progress based on the additive or additive × additive type of gene action, population improvement appears to be a hopeful alternative. The diallel selective mating system (Jensen, 1970) is a sound technique, which delays the quick fixation of gene complexes, permits the breakdown of linkage, and generally fosters the recombination and concentration of favourable genes/gene complexes into the central gene pool by a series of multiple crosses.

Normally the SCA effects do not contribute tangibly to the improvement of self-fertilizing crops, except where the commercial exploitation of heterosis is feasible. The SCA effects represent the dominance and epistatic interaction, which can be related with heterosis. However, in self-pollinated crops like barley, the additive × additive type of interaction component is fixable in later generations. Breeders therefore have a vested interest in obtaining transgressive segregants through crosses and producing more potent homozygous lines. Jinks and Jones (1958) emphasized that the superiority of the hybrids might not indicate their ability to yield transgressive segregants, and that SCA would provide a more satisfactory criterion.

The estimates of specific combining ability (SCA) revealed that, out of 45 crosses, 20 crosses in the F_1 and 19 crosses in the F_2 were good specific combiners for grain yield. It is noteworthy that 17 crosses showed positive and significant SCA effects for grain yield in both the F_1 and F_2 generations. The generation effects were also noticed in the SCA effects of the crosses. The highest positive significant SCA effect was exhibited by the cross RD 2503 × RD 2585 in both the generations. Other good combinations, which showed significant SCA effects for grain yield in both the generations, were RD 2035 × RD 2052, RD 2035 × BL 2, RD 2052 × BL 2, RD 2508 × RD 2552, RD 2552 × RD 2585 and RD 2052 × RD 2552 in both the F_1 and F_2 generations. These crosses were high yielders and in most of the crosses one of the parents involved was a good combiner, indicating that such combinations can be expected to produce desirable transgressive segregants. All the best crosses for grain yield also showed average to high SCA effects for most of the yield components. It is therefore recommended that new materials should be used in future breeding programmes for recombining the desirable traits in the envisaged elite genotypes.

Table 3
Estimates of general and specific combining ability effects for different characters of barley

Parent	Days to heading		Plant height		Tillers/plant		Flag leaf area		Spike length	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1	0.867*	-2.456**	-1.841*	-2.007**	0323**	0.235*	0.431**	0.319**	-0.138	-0.107
P_2	-3.106**	-3.122**	-3.433**	-3.219**	0.417**	0.394**	0.434**	0.406**	0.279**	0.277**
P_3	1.200**	-0.650	-1.064	-1.008	0.384**	0.393**	0.363**	0.363**	0.168*	0.134
P_4	-2.689**	-0.178	-3.253**	-3.209**	-0.291**	-0.245*	-0.310**	-0.255*	0.254**	0.227**
P_5	0.172	1.072*	-1.594*	-1.934**	-0.527**	-0.519**	-0.561**	-0.538**	-0.093	-0.081
P_6	1.617**	1.378**	9.761**	9.591**	0.053	0.101	-0.124	-0.057	0.041	0.014
P_7	1.756**	1.822**	-1.797*	-1.790*	-0.274**	-0.322**	-0.233*	-0.306**	-0.225**	-0.227**
P_8	0.089	-0.150	1.978**	1.918**	0.245**	0.264*	0.536**	0.518**	0.034	0.016
P_9	- 0.467	0.906*	-1.009	-0.786	-0.222*	-0.169	-0.332**	-0.257**	-0.241**	-0.262**
P_{10}	0.561	1.378**	2.251**	2.443**	-0.108	-0.132	-0.204*	-0.193*	-0.078	0.009
$SE(g_i)\pm$	0.295	0.372	0.574	0.583	0.077	0.082	0.072	0.077	0.067	0.067
$SE(g_i\text{-}g_j)\pm$	0.439	0.555	0.855	0.869	0.115	0.122	0.108	0.115	0.100	0.100
Parent	No.of spikelets/spike		No. of grains/ spike		1000-grain weight		Harvest index		Grain yield/plant	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1	0.751	0.143	0.600	0.051	1.585**	1.616**	0.876**	0.639*	1.587**	1.072**
P_2	2.243**	2.610**	2.245**	2.843**	1.638**	1.409**	0.888**	0.763**	2.316**	2.215**
P_3	2.314**	2.386**	2.239**	2.543**	1.309**	1.450**	0.445	0.735**	1.833**	2.237**
P_4	0.878	0.636	0.806	0.540	1.675**	1.519**	-0.077	-0.368	0.200	-0.093
P_5	0.031	-0.348	0.003	-0.547	-1.562**	-1.894**	-0.862**	-0.972**	-1.879**	-1.701**
P_6	-1.552**	-1.306*	-1.428**	-0.991*	-1.797**	-1.378**	-0.237	-0.059	-1.276**	-1.109**
P_7	-4.032**	-3.846**	-3.695**	-3.819**	-0.779**	-0.865**	-0.384	-0.563*	-2.125**	-2.065**
P_8	-0.022	-0.175	-0.247	-0.796	-0.585*	-0.558*	0.476	0.330	0.622**	0.494*
P_9	-0.614	-0.246	-0.694	-0.166	-0.027	0.135	-0.493*	-0.237	-0.711**	-0.614**
P_{10}	0.004	0.146	0.172	0.343	-1.456**	-1.434**	-0.632*	-0.269	-0.508**	-0.436*
$SE(g_i)\pm$	0.466	0.480	0.386	0.424	0.213	0.239	0.217	0.202	0.150	0.159
$SE(g_i\text{-}g_j)\pm$	0.694	0.716	0.576	0.633	0.318	0.357	0.323	0.301	0.224	0.238

P_1= RD 2035, P_2= RD 2052, P_3= RD 2503, P_4= RD 2508, P_5= RD 2552, P_6= RD 2585, P_7= RD 387, P_8= BL 2, P_9= ISBYT 4 and P_{10}= ISBYT 17.* and ** Significant at the 5 and 1% levels, respectively.

It is noteworthy that crosses which exhibited consistently positive SCA in both generations, also exhibited positive significant heterosis. Thus, the results of the present study indicated some relationship between SCA effects and heterosis. It is therefore suggested that SCA performance should be considered as a criterion for selecting the best crosses in barley. It may also be worthwhile to attempt bi-parental mating in the segregating generation among selected crosses to permit superior recombinations. All the important crosses involving parents with high × average, average × average and average × poor general combiners indicated that the non-additive type of gene actions, which are unfixable in nature, were involved in selected cross combinations.

The study demonstrates that both the additive (fixable) and non-additive (non-fixable) components of genetic variance were involved in governing the inheritance of almost all the quantitative traits, although additive genetic variance was predominant. Therefore, bi-parental mating and/or diallel selective mating, which allows the intermating of selected lines in different cycles and exploits both additive and non-additive gene effects could be useful in the genetic improvement of the characters of barley. The inclusion of F_1 hybrids showing high SCA, having parents with good GCA, in multiple crosses, could also prove a worthwhile approach for tangible advances in grain yield in six-rowed barley.

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STUDY OF THE FROST TOLERANCE AND WINTER HARDINESS OF EMMER (*TRITICUM TURGIDUM* SSP. *DICOCCON*) GENEBANK ACCESSIONS UNDER NATURAL AND ARTIFICIAL CONDITIONS

G. KOVÁCS, A. F. BÁLINT and J. SUTKA

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The growing interest in emmer cultivation has no doubt been stimulated by the increasing demand for traditional foods with an image of naturalness, especially on the organic market. The new economic situation could stimulate the breeding and production of emmer as the source of an especially valuable foodstuff. It is the task of breeders to produce emmer varieties that can survive even the hardest winter occurring in the targeted cultivation area without serious damage. The best sources to improve the winter hardiness are probably the emmer genetic resources stored in genebanks. Unfortunately no public data are available on the frost tolerance and winter hardiness of the various genebank accessions. In the present research the frost tolerance and winter hardiness of 10 winter emmer genebank accessions were studied under nursery and phytotronic conditions. The results suggest that the majority of the populations studied are frost-sensitive, and only few landraces have an acceptable level of winter hardiness and frost resistance.

Keywords: emmer, genebank accessions, frost tolerance, winter hardiness

Introduction

Emmer, the under-utilised tetraploid hulled wheat (*Triticum turgidum* ssp. *dicoccon*), was one of the first cereals ever domesticated. Farmers had it in their fields perhaps as far back as 12,000 years ago, and for several thousand years it remained a major cereal throughout the Middle East, Europe and North Africa. Then people switched to durum wheat (*Triticum turgidum* ssp. *durum*) which probably originated from emmer by mutation. Farmers preferred durum because its grain was free threshing, and during the past 2,000 years or so the older form, emmer, has fallen into oblivion. The cultivation of emmer has only survived in a few marginal mountain areas, remaining unknown to the general public until about twenty years ago. Recent surveys classified emmer as a crop seriously threatened by extinction, its use being strictly limited to marginal animal feeding systems (Perrino and Hammer, 1984). However, further investigations (D'Antuono and Pavoni, 1993; Mariani, 1994; Merezhko et al., 1996) indicated that the cultivation of emmer, although of limited size, was still present in many areas of Europe, where its use as a human food had solid roots in local tradition. Such traditional uses have been the starting point of renewed interest in this ancient tetraploid wheat. The growth of today's emmer cultivation may also have been stimulated by the increasing demand for traditional foods with an image of naturalness, especially on the organic market. The new economic situation could stimulate the breeding and production of emmer as the source of an especially valuable foodstuff.

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The antiquity of emmer and the wide area of its cultivation in the past have led to a large diversity of ecological types. The existing forms of emmer are clearly divided into four distinct groups, differing in morphological structure and in their combination of biological and agronomic characters (Merezhko et al., 1996). According to their origin, these groups are named Ethiopian, Eastern (Asian), European and Moroccan. The majority of emmer genetic resources are spring forms; accessions with winter and semi-winter growth habit occur only among materials originating from Central and Eastern Europe. According to previous results, the winter and semi-winter forms of emmer are characterised by the prevalence of forms with a short vegetation period, the fast formation and ripening of grains with a high protein content, high resistance to most wheat diseases and pests, and sufficient drought and cold tolerance to survive mild to moderate winters (Merezhko et al., 1996). Based on such previous studies, emmer is considered as a low input cereal with high ecological value for cropping systems under cold, wet climatic conditions. To successfully grow competitive emmer wheat under cool, wet climatic conditions, it is necessary to develop winter type emmer with good winter hardiness and frost tolerance. Unfortunately no published data are available to breeders on the winter hardiness and frost tolerance of different emmer genetic resources.

Frost tolerance is one component of winter hardiness. Seedlings which are frost tolerant generally survive the winter well, so that the study of frost tolerance gives a good indication of winter hardiness.

Under cool, wet climatic conditions the analysis of winter hardiness is only possible in the nursery every five to ten years. In mild winters it may prove impossible to detect any difference between the different accessions, while in severe winters the whole experiment may be destroyed (Fowler and Gusta, 1977). It is practically inconceivable for climatic factors to be reproduced year by year in the nursery. Since the evaluation of winter hardiness in the field may also be complicated by factors such as disease, flooding, soil moisture content, ice layer formation, and uneven snow cover, even in an average year, it is almost impossible to detect genetic differences between different genebank accessions. Frost tolerance tests under controlled climatic conditions, on the other hand, are highly reproducible and in the case of bread wheat they give a highly significant correlation with winter hardiness (Sutka et al., 1986). During the past two decades a wide range of methods for testing the frost resistance of different cereal species under controlled environmental conditions has been elaborated in Martonvásár (Sutka, 1981; Beke and Sutka, 1983; Veisz, 1987).

The aim of the present study was to study the frost tolerance of winter and semi-winter emmer genetic resources under controlled environmental conditions. The present paper also gives an account of the correlation obtained between the frost resistance and winter hardiness of emmer under nursery and phytotronic conditions.

Materials and methods

In the present experiment the winter hardiness and frost tolerance of ten emmer (*Triticum turgidum* ssp. *dicoccon*) genebank accessions were evaluated. Out of the 10 emmer accessions studied in this experiment, two (MvGB 302 and MvGB 348) were previously characterised as spring types. The other eight accessions (semi-winter type: MvGB 300 and MvGB 301; true winter type: MvGB 135, MvGB 304, MvGB 317, MvGB 349, MvGB 503 and MvGB 506) showed a winter or semi-winter growth habit, according to previous nursery data (Kovács, 1997-98, unpublished data). Winter hardiness was tested in the nursery, while the frost tolerance studies were carried out under controlled environmental conditions. Two bread wheat (*Triticum aestivum* ssp. *aestivum* L.) genotypes (Cheyenne and Chinese Spring) with known levels of frost resistance and winter hardiness (Sutka et al., 1986) were used as a control when testing the frost resistance and winter hardiness of emmer.

To test winter hardiness, the ten emmer accessions were sown, together with the control bread wheat varieties, in medium hard soil in the institute nursery on October 12th 2002. The experiment was set up in a complete random block design with three replications. The numbers of germinated seeds were counted on November 20th in the same year, while survival was scored the next March, and winter hardiness was expressed as percentage survival in each replication.

The method developed in the Martonvásár phytotron for the testing of bread wheat was used for the study of frost tolerance. Germinated seeds were sown randomly in wooden boxes with inner dimensions of 42 × 31× 18 cm. The growing medium was a 2:2:1 mixture of garden soil, humus mixture and sand. Fifteen rows, each consisting of 10 germinated seeds, were sown in each box. Each row contained a single genotype and the 15 accessions found in each box were taken as one replication. The experiment was carried out in 8 replications.

After germinating for two days in Petri dishes the emmer and bread wheat seeds were planted out in the boxes and watered with tap water for a week, after which they were given nutrient solution. During the preliminary growth period the quantity of irrigation water was gradually reduced to ensure low soil moisture content for freezing. The boxes were not watered during the course of the frost test. After freezing, the plants were again provided with a plentiful supply of water and nutrients.

The preliminary growth and hardening of the plants were carried in an autumn-winter type of plant growth unit according to Sutka and Veisz (1988). The preliminary growth period lasted for 5 weeks with decreasing temperature and illumination. During the 6th and 7th weeks hardening was carried out at a day temperature of +2°C and a night temperature of 0°C with 20-hour illumination. After hardening, the boxes were transferred to the frost resistance testing chamber, where the temperature was reduced by 1°C/h to a value of –4°C. Hardening was continued in this chamber for a further 2 days in the dark, after which frost treatment was carried out at –12°C for 24 hours. After thawing for 2 days at 0°C the frozen leaves were removed to promote shoot growth and avoid fungal infection. The plants were transferred to a growth bench, where they were grown for three weeks under optimal conditions (17°C day and 15°C night temperature, 14 hours illumination at 260 μm s^{-1} m^{-2} light intensity). Frost tolerance was assessed in terms of regrowth on a 0 (dead) to 5 (undamaged) scale and also as percentage survival. Statistical analysis was carried out using the SPSS 8.0 statistical software package.

Results and discussion

In order to be able to evaluate the nursery data it is necessary to describe the temperatures and the quantity of precipitation in the season 2002/2003. The air temperature dropped to -14°C in early December, followed by a slightly warmer period for around ten days, after which it dropped again to as low as –16°C at the end of the month. In mid-January the mean temperature was extremely low, with a minimum of below –23°C. The severe cold spell continued throughout February, resulting in an average temperature of –8 °C. Temperatures remained below zero until around 10th March. The precipitation was also extreme. During December, there was practically no snow cover on the nursery field. Only in early January was total snow coverage recorded, after which snow continuously covered the soil until the end of February. There was another cold spell in the first half of March. This was the coldest winter recorded over the last five years with the lowest quantity of precipitation. Judging by the meteorological data, it can be concluded that this winter was extremely hard for the plant material investigated. The winter hardiness values found for the experimental materials are shown in Figure 1.

According to the data of the bread wheat varieties, it can be seen that the winter of 2003 was a very rare year, which only occurs in every five to ten years under Hungarian climatic conditions. Of the two bread wheat varieties studied, only the frost-tolerant variety, Cheyenne survived this severe winter. The spring variety Chinese Spring was completely killed. Similar results were obtained in the case of emmer. Out of the 12 emmer accessions studied under field conditions the majority were totally destroyed by the frost. Only three winter emmer accessions (MvGB 304, MvGB 317 and MvGB 349) survived, while all the rest were killed by the extremely hard winter. Naturally, the winter-hardy wheat genotype Cheyenne showed the highest level of survival, followed by the emmer landraces MvGB 349 and MvGB 317, while the winter hardiness of the emmer accession MvGB 304 was significantly weaker. A high level of frost damage is understandable in the case of spring and semi-winter types, but was completely unexpected when true winter types were studied. Out of the five true winter type emmers, MvGB 506 and MvGB 135 exhibited no winter hardiness under nursery conditions.

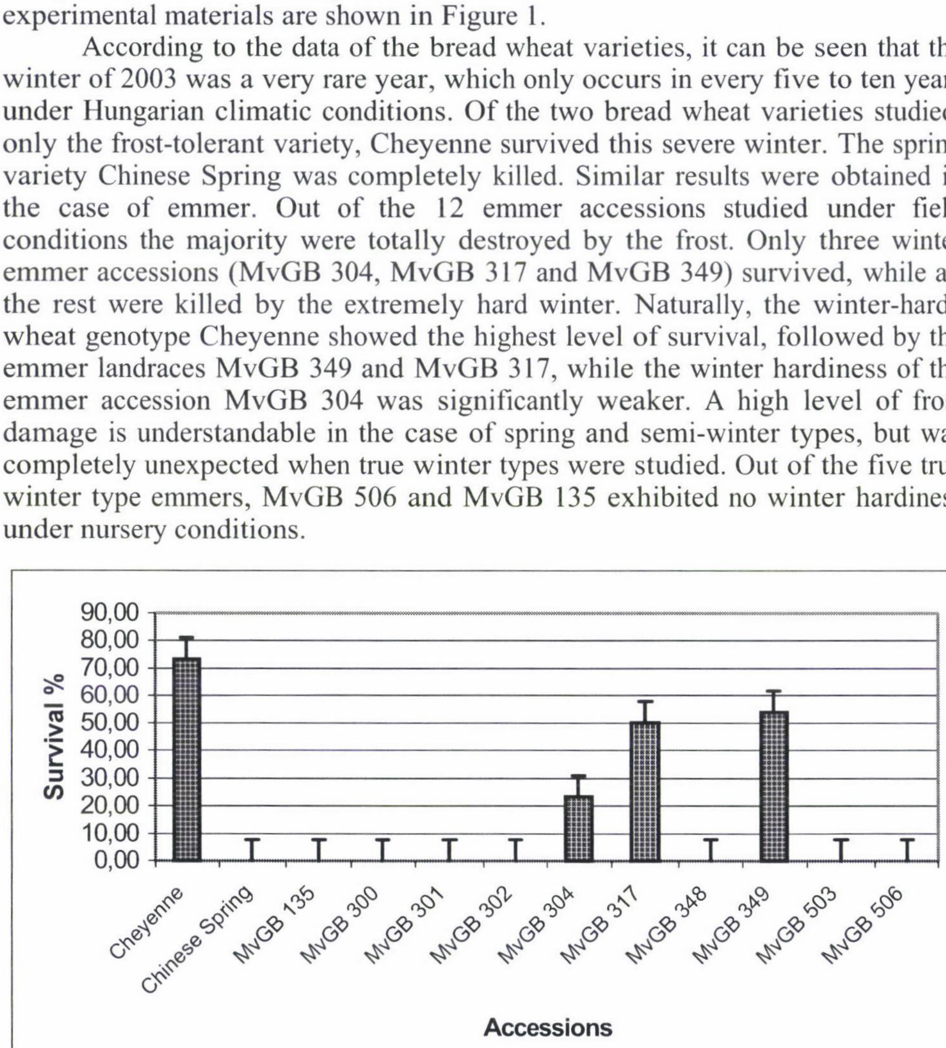

Fig. 1. Winter survival percentage of bread wheat varieties and emmer accessions in the nursery (2002/2003)

The results of the artificial freezing test are presented in Figure 2. According to the data obtained, all the emmer accessions survived the freezing temperature applied in the artificial freezing test, but significant differences could be observed. Under artificial conditions the frost tolerant Cheyenne and one winter emmer accession (MvGB 347) showed practically 100% survival. The MvGB 317 emmer accession also showed an acceptable level of survival. The spring variety Chinese Spring showed a significantly lower survival rate together with the other emmer accessions studied in this experiment.

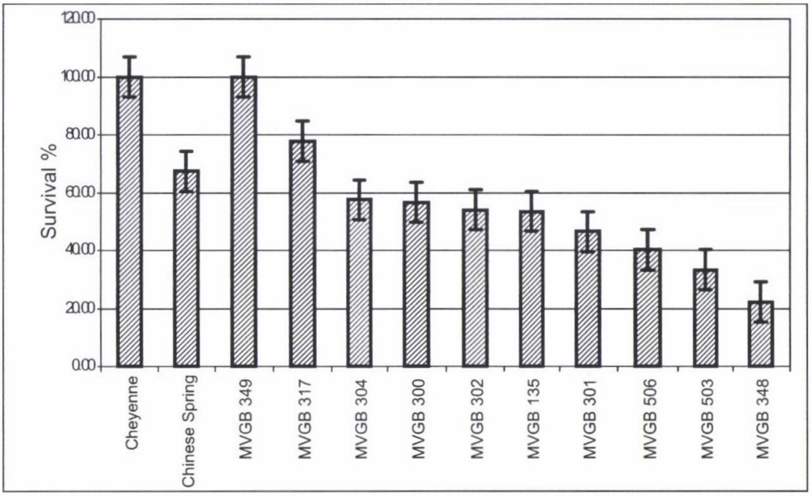

Fig. 2. Survival rate of the different emmer accessions after freezing at –12°C in a controlled environment (I - represents the significant difference at the p=0.95 probability level)

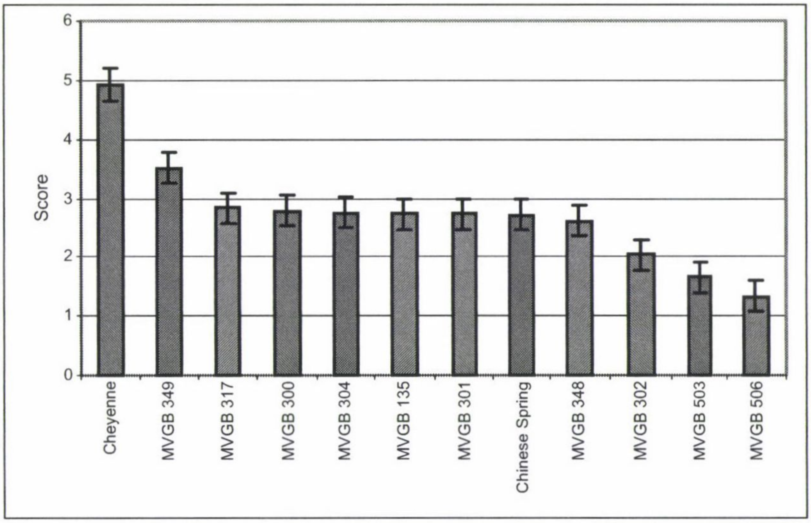

Fig. 3. Average frost resistance of emmer accessions assessed on a regrowth scale (I - represents the significant difference at the p=0.95 probability level)

These two winter emmer landraces also showed the best survival rate under natural conditions. However, MvGB 304, which survived the severe winter under field conditions, did not give good results in the artificial freezing test. Nearly 40% of the plants of this accession were killed even at –12°C. According to the data obtained in the artificial freezing test, the highest level of variance was observed for this population, suggesting that it may be a mixture of frost-tolerant and sensitive plants. The other emmer accessions showed weak frost tolerance, but in all cases some plants survived freezing.

The evaluation of regrowth on a 0–5 scale after freezing at –12°C shows that there is a highly significant difference between the frost-tolerant bread wheat variety Cheyenne and Chinese Spring. Such significant differences were not found for percentage survival, indicating that the analysis of regrowth is a better way of distinguishing different levels of frost tolerance under artificial conditions. This is in agreement with previous results (Sutka, 1981), suggesting that the method could be successfully used to test the frost resistance of emmer. According to the data, the accession MvGB 349 had the highest level of frost tolerance, significantly better than that of the other *Triticum dicoccon* samples. There were no significant differences between Chinese Spring and the other emmer populations, except for MvGB 506, which appeared to be the most frost-sensitive population in the studies. In general it can be assumed that the frost tolerance of the emmer accessions is not good enough to tolerate the hard winters experienced in Hungary.

When comparing phytotronic frost testing and nursery winter hardiness it can be seen that Cheyenne, MvGB 349, MvGB 317 and MvGB 304 showed the best performance in both environments. Considerable differences were also observed, however. The extremely hard winter killed practically all the genotypes with moderate frost tolerance, which were able to survive freezing at –12°C in a controlled environment. The great differences between the lowest temperature with a long cold period in the field and the moderate freezing temperature applied in the artificial freezing test caused higher selective pressure on the plant populations under nursery conditions. Probably due to these differences only a medium correlation could be demonstrated between the survival percentages obtained under natural and artificial conditions ($r = 0.57$).

Unfortunately no published results are available on the winter hardiness and frost tolerance of emmer genetic resources, so it is difficult to discuss the results obtained in the present experiment. It can be concluded that the level of frost resistance in emmer is similar to that of winter durum wheat, which is not enough to allow it to be grown competitively under cool, wet climatic conditions. It will thus be necessary to improve the frost resistance of high-yielding winter emmer breeding material with the help of highly frost-tolerant genetic resources of emmer.

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ELECTRON MICROSCOPE STUDIES ON COLD PRETREATED AND NON-PRETREATED ANTHERS OF MAIZE (*ZEA MAYS* L.) GENOTYPES WITH DIFFERENT EMBRYOGENIC CAPACITIES

É. SZAKÁCS and B. BARNABÁS

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The relationship between ultrastructural changes in the anthers caused by cold pretreatment and the haploid induction capacity of different maize genotypes was investigated. The degeneration of the tapetal cells appears to be genotype-dependent, but the extent of the degeneration is not correlated with the androgenetic ability of the given genotype. Based on the stainability of the cytoplasm, two microspore types were found. The results suggest that the "dense" microspores take part in pollen embryogenesis.

Key words: anther culture, cold pretreatment, electron microscopy, maize

Introduction

The tapetal layer of the anther wall plays a very important role in the normal gametophytic development of the pollen grains. Among other things, it provides the developing microspores with nourishment and takes part in the construction of the pollen wall.

In the scientific literature data on the role of the anther wall in pollen embryogenesis are few and contradictory. In microspore cultures of *Datura innoxia* (Nitsch and Norreel, 1973) and *Hordeum vulgare* (Sunderland et al., 1982) the culture medium had to be conditioned with anthers or extracts of anthers for successful haploid induction. It was thus suggested that some kind of conditional factor had been produced and released into the medium by the anthers. Tyagi et al. (1979) described just the opposite effect of the anther wall in isolated pollen cultures of *Datura innoxia,* since it was necessary to change the medium frequently to achieve induction (Sunderland and Roberts, 1979). This observation suggested that inhibitory substances had been released from the anthers. In *Nicotiana tabacum* anther cultures, an analysis of the amino acids in the anther wall demonstrated that the level of serine (Nitsch, 1974) and glutamine (Horner and Pratt, 1979) had rapidly increased, so the role of the anther wall is probably to produce these amino acids and to transmit them to the microspores.

It is well known that in the case of maize microspore embryogenesis can be induced only after cold pretreatment of the tassels (Genovesi and Collins, 1982; Dieu and Beckert, 1986). Genovesi and Collins (1982) found that two types of anther appeared in the culture following the pretreatment. One was dark yellow and thin and produced many more embryos than the other type, which was light yellow and swollen. On the basis of all these data the question arises of whether the cold pretreatment which promotes haploid induction only causes biochemical changes in the anthers or whether structural changes can also be detected in the anther tissues, and whether these changes are related to the haploid induction capacity of the given maize genotype. This question is studied in the present paper.

Materials and methods

Two doubled haploid maize lines (DH5 and DH7) with moderate *in vitro* androgenic capacity derived from anther culture, their single crossed hybrid (DH5 × DH7) with high androgenic capacity and two non-androgenic inbred lines (A188 and B73) were used in the present study. The maize plants were grown in the experimental nursery from May to September. The tassels were removed from the donor plants prior to their emergence from the leaf whorls. Anthers from the bottom, middle and upper parts of the main axis and side branches were used to check the microspore stages using Alexander's (1969) staining procedure. The mid-uninucleate microspore stage was chosen for the analyses.

For cold pretreatment, the tassels were wrapped in aluminium foil and stored in a 7°C refrigerator for 7 days. Some of the pretreated anthers were cultured on G induction medium (Genovesi and Collins, 1982) so as to check the effectiveness of the cold treatment. The cultures were kept at 14°C in the dark for 14 days, then at 28°C in the light for 4–6 weeks. The remaining cold-treated anthers and the control samples (without cold pretreatment) were fixed for light and electron microscope studies. They were dissected from the tassels, cut into three or four parts, immersed in a solution of 3% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2) and stored at 4°C overnight. Following three rinses in the same buffer for one hour each, the material was post-fixed in 1% cacodylate-buffered osmium tetroxide for 2 h at 4°C, then rinsed twice in the buffer for 15 min and once in distilled water for 30 min. After dehydration in a graded series of ethanol, the individual anther segments were embedded in Spurr's resin (Spurr, 1969). Thin (1 μm) and ultrathin (95–100 nm) sections were cut with glass knives using a Reichert-Jung Ultracut E microtome. The thin sections were stained with basic toluidine blue dye. The ultrathin sections were collected on mesh grids and stained with 7% uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Observations were carried out with a Hitachi UH-12 transmission electron microscope.

Results

1. Effect of cold pretreatment on anther response frequency

The results recorded for anther cultures set up to check the efficiency of the cold pretreatment correlated well with the data reported by Barloy et al. (1989). No pollen embryogenesis was observed for inbred lines A188 and B73, doubled haploid lines HD5 and HD7 gave anther responses of 38.0% and 28.0%, respectively, and the hybrid HD5 × HD7 showed the highest (56.0%) anther response.

2. Ultrastructure of the anther wall before cold pretreatment

Prior to the cold pretreatment, the somatic tissues of the anther walls were in the same stage in all the genotypes (Fig. 1). The epidermal cells were vacuolized, but though they contained little cytoplasm, the cell organelles were clearly recognizable. The structure of the endothecial cells was very similar to that of the epidermal cells, except that the amyloplasts contained more and larger starch grains. The tapetal layer consisted of closely packed square cells with large nuclei. These cells were characterised by very dense, darkly stained cytoplasm rich in cytoplasmic organelles, particularly in endoplasmic reticula and mitochondria, indicating that they were highly active. A number of small vacuoles and lipid droplets could also be observed.

3. Ultrastructure of the microspores before cold pretreatment

Two types of microspores, “dense” and “clear”, were distinguishable after staining the semi-thin sections (Fig. 2). Cytoplasm of the “clear” type was only faintly stained, in contrast with that of the “dense” type, which was coloured very intensely. This difference between the two pollen populations was confirmed by electron microscopy. The cell organelles of the “clear” microspores were clearly recognizable and dispersed in the cytoplasm, which contained vacuoles of various size (Fig. 3), while the chromatin in the nucleus showed slight electron opacity. In the "dense" microspores the organelles could not be identified with the exception of the proplastids (Fig. 4). Although vacuoles were present in the cytoplasm, they were fewer and smaller than in the “clear” microspores. The staining of the nucleus and the cytoplasm showed almost the same intensity. The pollen wall contained numerous “channels”.

The possibility that the “dense” microspores were defective or had died was excluded by a fluorochromatic reaction (FCR) test. The frequency of viable microspores was much higher (99%) than expected from the ratio of the two microspore populations.

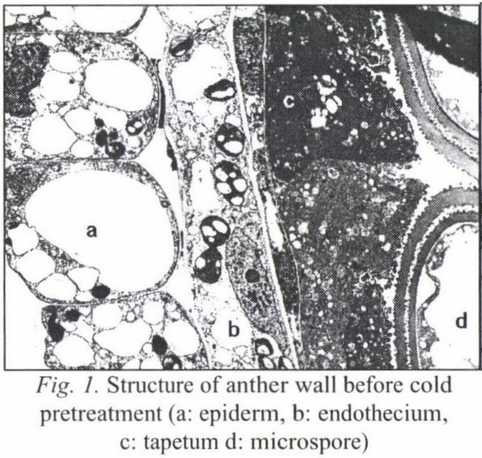

Fig. 1. Structure of anther wall before cold pretreatment (a: epiderm, b: endothecium, c: tapetum d: microspore)

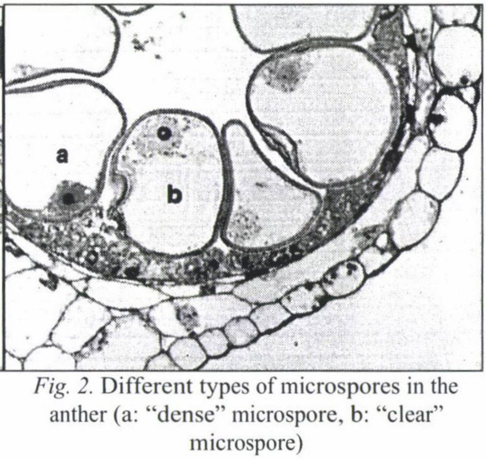

Fig. 2. Different types of microspores in the anther (a: “dense” microspore, b: “clear” microspore)

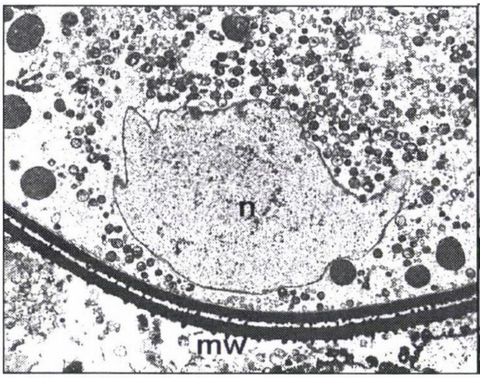

Fig. 3. "Clear" microspore (mw: microspore wall, n: nucleus)

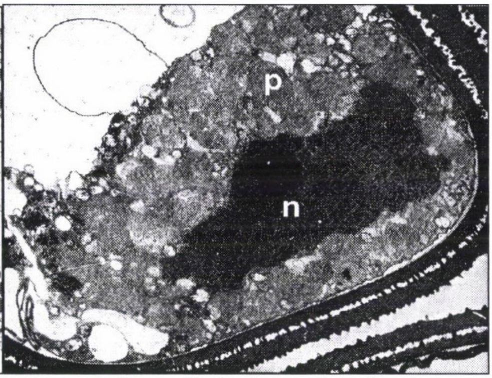

Fig. 4. "Dense" microspore (n: microspore nucleus, p: proplastids)

4. Ultrastructure of the anther wall after cold pretreatment

After cold pretreatment for 7 days the only change observable in the epidermal cells was the disappearance of the starch content from the plastids. A similar change was found in the endothecium, in addition to which the structure of the plastids altered. The grana were replaced by prolamellar bodies (Fig. 5). The response of the tapetal layer to the pretreatment depended on the genotype. In hybrid HD5 × HD7 the tapetal cells kept their original shape and their cytoplasm remained active, as indicated by the large quantity of cell organelles. Only the increase in the number and size of the vacuoles suggested a slight degeneration of the tapetum. The tapetal cells of inbred line A188 showed the greatest changes. They lost their original shape and the cytoplasm disappeared almost completely. The remnants of the cells were either filled with lipid or consisted of the cell wall and a large vacuole (Fig. 6). The degree of degeneration in the other genotypes (B73, HD5, HD7) ranged between that of HD5 × HD7 and A188.

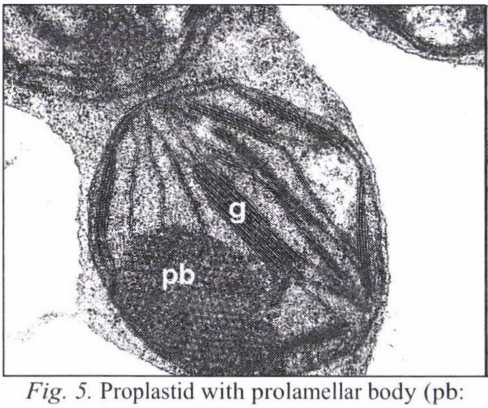

Fig. 5. Proplastid with prolamellar body (pb: prolamellar body, g: granum)

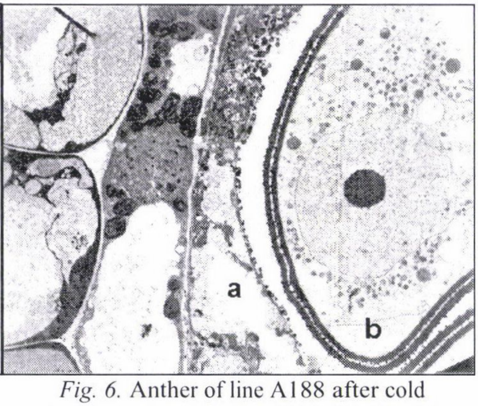

Fig. 6. Anther of line A188 after cold pretreatment (a: tapetum with large vacuoles, b: "clear" microspore)

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5. Ultrastructure of the microspores after cold pretreatment

The “clear” and “dense” microspores observed in non-pretreated anthers could also be found after the cold pretreatment. The difference between the "dense" microspores in pretreated and non-pretreated anthers was that a highly contrasted deposition was present on the tonoplast, the membrane of the vacuole, after the pretreatment (Fig. 7). This was absent in the “clear” microspores. Large pores were seen on the nucleus membrane of the “dense” microspores indicating the activity of the nucleus (Fig. 8).

The ratio of “dense” microspores (“dense” microspores/“dense” + “clear” microspores) before and after cold pretreatment, and the anther response of the five maize genotypes are presented in Table 1.

“Dense” microspores were observed at the lowest frequency in the two inbred lines: 4% before and 3% after pretreatment in the case of A188 and 5% and 6% in the case of B73. The largest proportion of “dense” microspores was found in hybrid HD5 × HD7, where their frequency was 54% before and 64% after pretreatment. The frequency of “dense” microspores in the doubled haploid lines showed intermediate values of 25% and 27% in HD5 and 21% and 19% in HD7. The values before and after pretreatment did not differ significantly from each other. A comparison of the ratio of “dense” microspores and the anther response showed a good correlation between them. In some genotypes, the accumulation of ferritin (a protein complex containing iron) was observed following the cold pretreatment; this was most pronounced in the doubled haploid line HD5 (Fig. 9).

Discussion

Many references can be found to the role of the anther wall in *in vitro* pollen embryogenesis (Nitsch and Norreel, 1973; Sunderland et al., 1982; Horner and Pratt, 1979), some of which emphasize the necessity of a cold pretreatment on the inflorescence of cereals, particularly in maize, before culturing the anthers (Genovesi and Collins, 1982; Dieu and Beckert, 1986). In the present paper the structural changes caused by cold pretreatment in the anther wall tissues and in microspores, and correlations between these changes and the androgenetic ability of different maize genotypes were studied. The degeneration of the tapetal cells after cold pretreatment, previously described by several authors (Cadic and Sangwan-Norreel, 1983; Sunderland et al., 1984), was also observed in the present study. In ultrastructural studies in rice anther culture Nakano and Maeda (1989) found that the initiation of *in vivo* autolysis in the tapetal cells was a crucial point in the induction of sporophytic development in the microspores. The present results do not give confirmation of this. The tapetum layer of hybrid HD5 × HD7, which gave the highest anther response, degenerated least while that of the non-androgenetic inbred line A188 degenerated to the highest degree. The tapetum layer of the other androgenetic and non-androgenetic lines (HD5, HD7, B73) showed a significant extent of degeneration. It thus seems that though the degeneration of the tapetal cells is genotype-dependent, the extent of degeneration is not correlated with the androgenetic ability of the given genotype.

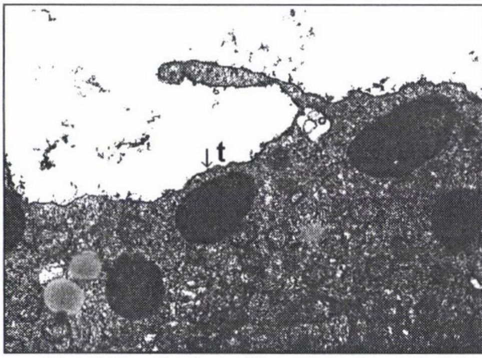

Fig. 7. Tannin deposition on the tonoplast (t: tonoplast)

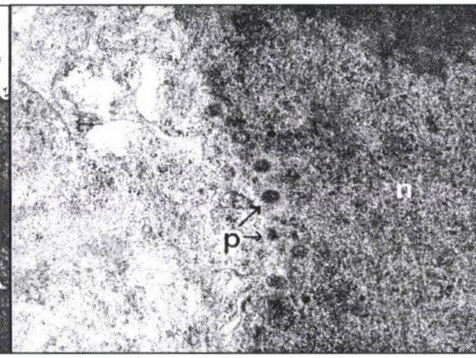

Fig. 8. Pores on the nucleus membrane (n: nucleus, p: pores)

The disassimilation of the starch grains in plant somatic tissues during cold treatment, observed in the present study, is a general phenomenon (Taylor and Craig, 1971; Sangwan and Sangwan-Norreel, 1987; Wilson et al., 1978). Nevertheless, it is possible that microspores deprived of a nutrient supply from the mother plant may satisfy their sugar needs from the starch in the pollen wall tissues. Consequently, sugar starvation, which is considered to be a trigger of *in vitro* androgenesis (Wei et al., 1986; Kyo and Harada, 1986), probably does not occur in the microspores.

Pollen dimorphism has only been described so far in the case of mature pollen grains (Heberle-Bors, 1985; Dale, 1975; Wenzel and Thomas, 1974; Horner and Street, 1978). In the present study a new kind of pollen dimorphism, microspore dimorphism, was observed. In contrast to "p-pollen" (Heberle-Bors, 1985) the "dense" and "clear" microspores did not differ in size, but their cytoplasmic structure and staining were different both at the light and electron microscope level. Sunderland (1978) also found slightly and intensly stained pollen populations in *Nicotiana*, but these were formed as the result of cold pretreatment. The newly discovered dimorphic microspores were present both prior to and after the cold treatment and their frequency did not change during the treatment. The strong stainability of the cytoplasm in the "dense" microspores is probably a consequence of the high cytoplasmic activity. High cytoplasmic activity was detected in *Nicotiana* anther cultures during the induction period (Dunwell and Sunderland, 1974) and intensely stained cytoplasmic RNA was reported in embryogenetic pollen grains of *Datura innoxia* (Sangwan-Norreel, 1978). This activity was thought to be due to the regression of the gametophytic cytoplasm (Dunwell and Sunderland, 1974). The decrease in the number of nuclear pores in the "dense" microspores may be correlated with a decrease in the transcriptional activity of the nucleus (Garrido et al., 1993). Previously reported cellular changes such as the regression of cytoplasmic contents, chromatin condensation and altered nuclear structure during pollen embryogenesis in *Nicotiana tabacum* (Garrido et al., 1995) were also observed in the present study. These changes seem to be prerequisites for the switch from gametophytic to sporophytic generation.

Table 1
Frequency of "dense" microspores before and after cold pretreatment for 7 days

Genotypes	Frequency of "dense" microspores (%)		Anther response (%)
	cold pretreatment 0 day	cold pretreatment 7 days	
A188	4.0	3.0	0.0
B73	5.0	6.0	0.0
HD5	25.0	27.0	38.0
HD7	21.0	19.0	28.0
HD5 × HD7	54.0	64.0	56.0

Another clearly perceptible difference between "clear" and "dense" microspores is that the tonoplast membrane of the "dense" microspores is covered with an electron-dense tannin deposition. Tannin deposition on the tonoplast, acting as a marker of embryogenetic pollen grains, was previously reported in members of the Solanaceae. In the studies of Sangwan and Camefort (1983) the frequency of *Datura metel* microspores having tonoplasts covered with a tannin layer correlated with the ratio of embryogenetic microspores, thus supporting the present results. It was suggested that the tonoplast became active when the tannin deposition began, but the biochemical background of this process is not known yet. It is possible that a biochemical reaction activating embryogenesis is generated in the course of the cold pretreatment and that tannin is an intermediary or final metabolic product. By contrast, Zaki and Dickinson (1990) found no correlation between the presence of tannin deposition and the frequency of haploid induction in *Brassica* anther culture. Nevertheless, on the basis of the present results it appears that the "dense" microspores take part in pollen embryogenesis, at least in the case of maize.

In general, ferritin occurs in plants with reduced photosynthetic activity or in plants which are not completely differentiated, so it is possible that the appearance of ferritin is due not to the cold treatment but to the effect of storing tassels in the dark.

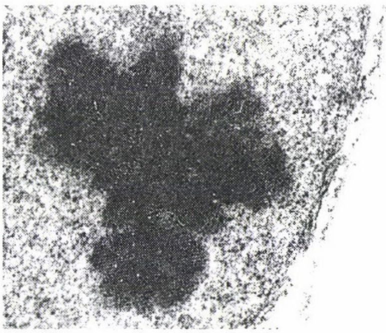

Fig. 9. Ferritin crystal in a proplastid

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INFLUENCE OF GROWTH FACTORS ON THE YIELD AND QUALITY OF DRY BEANS

E. NEMESKÉRI[1] and L. NAGY[2]

[1]AGRONA LTD., DEBRECEN, HUNGARY
[2]RESEARCH INSTITUTE FOR FISHERIES, AQUACULTURE AND IRRIGATION, SZARVAS, HUNGARY

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Investigations were made on the relationship between plant density and plant height, and on the yield, thousand seed mass, and ratio of diseased and broken seeds of varieties with different seed sizes. Experiments were carried out to analyse the effects of potassium on the yield, bacterial diseases and nutritive quality of the seed of bean varieties. Three dry bean cultivars representing the small, medium and large seed size groups were investigated. Six plant density treatments were chosen based on theoretical seed norms, taking the germinative value of the seeds into consideration. The correlation between plant density and yield average showed that the volume of yield increased for varieties with large and medium-sized seeds up to a plant density of 285–400 thousand/ha, after which it declined. On the basis of the results, yield averages at plant densities of 285–334 thousand plants/ha were 0.17 t/ha higher than those achieved at low density (200 thousand plants/ha). At greater plant density the plant height increased in the case of large-seeded varieties and there was a considerable decrease in the thousand seed mass. With an adequate water supply a high level of potassium decreased the number of pods and seeds per plant compared with the basic level, which gave a yield of 2.5 t/ha. The use of high rates of potassium fertilizer decreased the number of infected seeds, but the differences were only significant for the small-seeded variety. A moderately high potassium level was advantageous for food quality, particularly during drought.

Key words: bean, yield quality, plant density, potassium fertilizer

Introduction

Legumes with large seed size, such as peas, beans and soybeans, are more sensitive to mechanical damage than cereals. A number of abnormal seedlings are observed due to the slight fracturing or cracking of the cotyledons during threshing. The ratio of abnormal seeds increased when there was a long dry period during seed development (Vieira et al., 1992) and in seeds stored at high temperature (Hernandez-Livera et al., 1990). Seed yield was decreased not only by high temperature but also by bacterial diseases caused by *Xanthomonas phaseoli.* In the case of serious bacterial infection the rate of aborted seeds within the pods increased, causing a significant decrease in the seed yield. Sangakkara (1989) and Sexton et al. (1994) found that genetic factors related to large seed size were responsible for the relatively low yielding ability of varieties with large seeds. After studying factors affecting growth and crop development, White and Gonzales (1990) stated that the size of the seed was correlated with the size of the cells closing stoma, the parenchyma of the

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cotyledons, the endoderm of the hypocotyl and the roots. The growth habit and t stem stability of the varieties cultivated in the field limited the plant density. Various seeding norms are needed for different seed sizes to ensure the optimum plant density. The use of seeding rates higher than the optimum increased the production costs, particularly in the case of beans with large seeds.

Potassium has been found to enhance yielding capacity, resistance to stress and diseases, and crop quality. Bergmann (1979) reported that potassium fertilizer increased or decreased the yield depending on the ratio of K/Mg in the soil. When this ratio was near 1 the yield increased, but when it was over 3 it caused a yield decrease. A high potassium content in the soil resulted in Mg and Ca deficiency in the plants, leading to a deterioration in the cooking quality. Under a high soil moisture regime, potassium increased the root dry weight and root hairs to a greater extent than when the plants were grown under dry conditions (Sangakkara et al., 1996).

In the present study, the optimum plant densities of dry bean varieties with various seed sizes were investigated. The aim was to reveal correlations between the plant density and the thousand seed mass and seed quality. The effects of potassium on the yield, bacterial diseases and nutritive quality of the seed of bean varieties were analysed.

Materials and methods

Experiment I

From 1997 to 1999 investigations were made to determine the growing space requirements of three dry bean cultivars representing the small, medium and large seed size groups. The field experiments were set up in four repetitions in a split-plot design on chernozem soil. The distance between rows was 50 cm and the row length 9 m, giving a plot size was 13.5 m^2. The group with small thousand seed mass (200 g) was represented by the white, round-seeded variety Debreceni Gyöngy. This variety grows into an erect bush with small leaves. The Békési Fehér bean variety, representing the white medium-sized seed group (300 g), had a strong stem with large leaves. The large-seeded (500 g) bush bean variety Debreceni Tarka also grows on an erect stem with large leaves. Six plant density treatments were chosen based on theoretical seed norms, taking the germinative value of the seeds into consideration (Table 1).

Each year the experiments were planted between 5 and 12 May and harvested using a plot-sized machine by 20 August, depending on the weather. Harvesting was begun when the moisture content of the seeds was 16%, except in 1999 when it was carried out 18%. Samples were taken from each replicated plot to determine the thousand seed mass and the ratio of diseased and broken seeds. The ratios of infected and broken seeds were expressed as a weight percentage. The quality of the seeds was characterized by the ratio of diseased and broken seeds, because these had an indirect effect on the quantity of abnormal and diseased seedlings.

Experiment II

In 1999 and 2000, experiments were carried out to investigate the utilization of potassium fertilizer by small- and large-seeded bean varieties (Debreceni Gyöngy and Debreceni Tarka) grown on chernozem soil rich in humus, well supplied with nitrogen and phosphorus, and with satisfactory levels of potassium. The plot size and experimental design were the same as those described for Experiment I. The fertilizer treatments were as follows: control, where the plants were grown without fertilizer; $K_{1.0}$:basic treatment (N: P_2O_5: K_2O = 1: 0.5: 1) providing sufficient potassium fertilizer for a yield of 2.5 t/ha. Based on soil analysis this quantity was N: 90, P_2O_5:

30, K_2O: 100 kg/ha. $K_{1.5}$: where the potassium fertilizer was increased to 1.5 times the basic treatment and $K_{2.0}$ where it was doubled. The nitrogen and phosphorus doses were constant in all the fertilizer treatments.

Ten plant samples were collected from each of the four replications, and the number of pods and seeds, the number of diseased seeds and the thousand seed mass were measured. The ratio of seed mass and seed coat mass was expressed as the seed coat %. The data were evaluated with two-factor ANOVA and regression analysis.

Results

Experiment I

In 1998 and 1999 the temperature and precipitation were favourable for seed development. There were no differences in yield or bacterial infection between the white bean varieties with small and medium-sized seed. Averaged over the years the small-seeded variety was found to have better yield stability than the medium-sized variety. Debreceni Tarka, which has large coloured seeds, exhibited a yield decrease of 50% compared with the other varieties, which could be attributed not only to its genetic background, but also to its susceptibility to *Xanthomonas phaseoli*. Many factors, such as the moisture content and size of the seed, the closeness with which the cotyledons are joined and the structure of the seed coat, have an influence on the extent of mechanical damage to the seeds. The large-seeded variety was found to be the most sensitive to mechanical damage, as the ratio of broken seeds was the largest for this variety (17–19%). The same tendency was observed when harvest took place at high seed moisture content.

The treatment with 200 thousand plants/hectare was considered as the control, and was equivalent to low plant density in seed production. According to the results, an increase in plant density enhanced the yield (Table 1). Averaged over the years, the yield was 0.17 t/ha higher at plant densities of 285 and 334 thousand plants/hectare than at low density (200 thousand plants/ha). At a plant density of above 285–334 thousand the yield surplus dropped by half (0.08 t/ha). The thousand seed mass is known to be controlled by genes, but nevertheless changed a little with the climatic conditions. An increase in plant density slightly decreased the thousand seed mass, but significant differences were only detected in 1998. An increase in plant density had no significant effect on the ratio of diseased seeds, though this ratio increased slightly at high plant density (400–450 thousand plants/ha), particularly in bean varieties susceptible to bacterial diseases. The results on the ratio of broken seeds were contradictory. The ratio was highest at plant densities of 285–334 thousand plants/ha, and decreased slightly at high plant density.

Table 1
Effect of plant density/ha on the production traits of bean varieties with different seed sizes

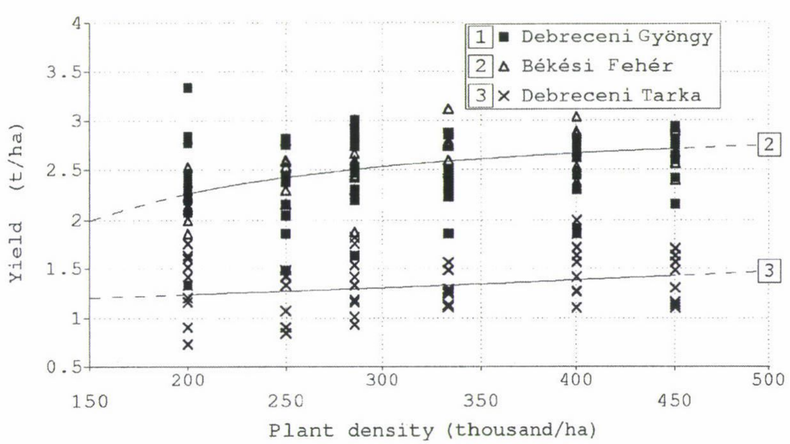
Trait	Plant density*	1997	1998	1999	Average
Plant height (cm)	200	59.31b	51.71	57.33d	56.12
	250		51.00	58.17cd	54.59
	285	61.76a	49.46	60.33bc	57.18
	334		51.29	61.25b	56.27
	400	62.95a	50.17	60.83b	57.98
	450		49.58	64.67a	57.13
	$LSD_{5\%}$	1.94		2.57	
Seed yield (t/ha)	200	2.16	1.93b	1.88b	1.99
	250		2.00ab	1.94b	1.97
	285	2.27	2.07ab	2.10ab	2.15
	334		2.16a	2.13a	2.15
	400	2.34	2.18a	2.18a	2.23
	450		2.19a	2.26a	2.23
	$LSD_{1\%}$		–	0.19	
	$LSD_{10\%}$		0.21		
Thousand seed mass (g)	200	363.48	340.75a	329.58	344.60
	250		337.75a	328.08	332.92
	285	348.14	335.08a	328.33	337.18
	334		333.42a	325.58	329.50
	400	341.90	335.25a	321.66	332.94
	450		326.67b	322.92	324.80
	$LSD_{10\%}$		7.71		
Bacterial seed infection (%)	200	8.88	12.21	6.58	9.22
	250		12.61	6.71	9.66
	285	8.66	13.57	6.47	9.57
	334		13.92	7.07	10.50
	400	7.13	14.71	6.32	9.39
	450		13.03	6.72	9.88
Broken seed (%)	200	12.31	11.54b	1.74	8.53
	250		11.99b	1.80	6.90
	285	12.08	14.45a	1.82	9.45
	334		13.39a	1.80	7.60
	400	11.91	12.80ab	1.67	8.79
	450		11.32b	1.56	6.44
	$LSD_{5\%}$		2.03		

*thousand/ha; Different letters within the columns indicate significant differences between the data pairs.

Investigations were made on the relationship between plant density and plant height, and on the yield, thousand seed mass and ratio of diseased and broken seeds in varieties with different seed sizes. Analysing all the data, a medium correlation (r = 0.55) between plant density and yield was only found for the Békési Fehér bean variety, representing the 300 g thousand seed mass group (Fig. 1). The yield of this variety increased to the evenly extent from 285 to 400 thousand plants/ha based on the Y' regression equation, but the thousand seed mass of this variety did not change at high plant density. There was no correlation between plant density and yield for the small-seeded (200 g) variety Debreceni Gyöngy. A weak correlation (r = 0.22) could be shown between the plant density and yield of the Debreceni Tarka variety having a thousand seed mass of 500 g. Calculated from the Y' regression equation, there was a slow increase in yield from 285 to 400 thousand plants/ha, after which it decreased. The weak correlation coefficient (r = 0.22) proved that the yielding ability of large-seeded varieties hardly increased with a rise in plant density (Fig. 1). At high plant densities, the plant height of the Debreceni tarka variety increased, but its thousand seed mass significantly decreased (Fig. 2). This means that when large-seeded varieties are grown at a density of over 334 thousand plants/ha, even under favourable climatic conditions, their thousand seed mass will decrease.

Experiment II

In 1999 the temperature and precipitation were favourable for pod setting and seed development in contrast with 2000, which was very dry. These different years gave a good demonstration of the effects of increasing potassium fertilizer on the yield and seed quality of beans grown at optimum plant density. According to the results, potassium did not influence the increase in bean yield. The variety Debreceni Gyöngy seemed to utilise high levels of potassium better for seed development than the large-seeded variety Debreceni Tarka (Table 2). The number of pods per plant was considerably larger in the treatments, compared with the control, due to the effect of potassium. Significant differences between the fertilizer treatments were only observed for the pod number/plant of the small-seeded variety. High rates of potassium ($K_{2.0}$), equivalent to 200 kg/ha active agents, decreased the pod number/plant and the seed number/plant of small-seeded beans, while for beans with large seeds no significant differences could be detected. The flowering time was prolonged and many pods were set as the result of high potassium rates, but few of them became mature. This was why the number of pods and seeds harvested decreased.



1	n=60	r=-0.2082	p=----
2	n=60	r=-0.5524	p=0.1% Y'=3.066097-161.516656*1/X
3	n=60	r=-0.2202	p=10% Y'=1/(0.895828-0.000432X)

Fig. 1. Effect of plant density on the yield of dry bean varieties with different seed sizes

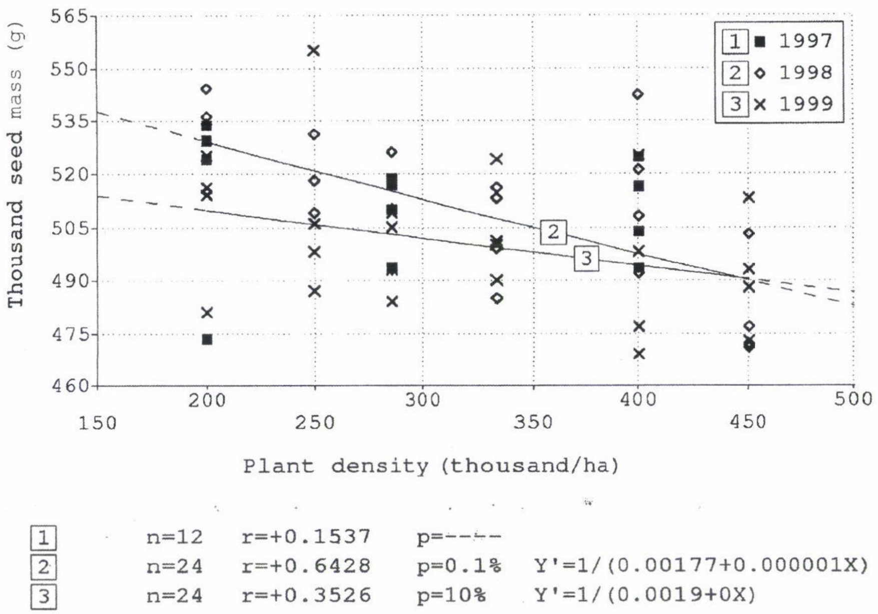


Fig. 2. Effect of plant density on the thousand seed mass of dry bean variety Debreceni Tarka

Table 2
Effect of potassium on the yield capacity of dry bean varieties

Variety	Treat-ments	Seed t/ha			Pods/plant			Seed/plant		
		1999	2000	Average	1999	2000	Average	1999	2000	Average
Debreceni Gyöngy	Control	2.39	1.81	2.10	20.95c	14.00	17.48	61.20c	23.00	42.10
	$K_{1.0}$	2.02	2.32	2.17	31.40a	12.80	22.10	105.80a	22.30	64.05
	$K_{1.5}$	2.22	2.23	2.23	31.70a	13.80	22.75	95.60ab	18.00	56.80
	$K_{2.0}$	2.43	2.30	2.37	27.20b	15.40	21.30	87.60b	23.60	55.60
Debreceni Tarka	Control	0.94	0.63	0.79	6.13e	4.90	5.52	15.00d	7.90	11.45
	$K_{1.0}$	1.31	0.84	1.08	8.65d	7.40	8.03	22.90d	10.50	16.70
	$K_{1.5}$	1.22	0.90	1.06	9.38d	8.20	8.79	20.50d	10.50	15.50
	$K_{2.0}$	1.15	0.63	0.89	7.58ed	4.20	5.89	18.70d	5.20	11.35
$LSD_{5\%}$		0.38	0.50		2.50	7.90		12.90	12.10	
$*LSD_{5\%}$		0.53	0.71		3.50	11.20		18.20	17.10	

*Differences between treatments; Different letters within the columns indicate significant differences between the data pairs.

Potassium is said to have a positive effect on the disease resistance and quality of crops. In the case of beans, the application of high rates of potassium fertilizer decreased the number of infected seeds, but significant differences could only be revealed in the small-seeded variety Debreceni Gyöngy (Table 3). In a dry year (2000) a larger protein content was measured in the seed than in 1999. The differences in protein content were only significant between the

Table 3
Influence of potassium on yield quality of dry bean varieties

Variety	Treat-ment	Infected seed/plant			Seed coat %			Protein %		
		1999	2000	Average	1999	2000	Average	1999	2000	Average
Debreceni	Control	15.5b	20.4	17.95	8.79	8.00	8.40	19.43b	21.86b	20.56
Gyöngy	$K_{1.0}$	28.5a	10.4	19.45	9.76	9.45	9.62	20.53ab	23.0ab	21.77
	$K_{1.5}$	21.1a	10.7	15.90	11.13	9.14	10.14	21.82a	25.48a	23.65
	$K_{2.0}$	9.2c	13.6	11.40	10.00	7.17	8.59	21.09ab	25.05a	23.07
Debreceni	Control	3.6d	6.0	4.80	10.54	10.58	10.56	19.97b	22.73b	21.35
Tarka	$K_{1.0}$	5.2cd	7.3	6.25	10.73	9.58	10.16	20.86ab	23.23ab	22.05
	$K_{1.5}$	6.8cd	9.5	8.15	11.39	9.05	10.22	21.49a	25.11a	23.30
	$K_{2.0}$	5.4cd	4.5	4.95	10.69	9.95	10.32	21.36ab	24.61ab	22.99
$LSD_{5\%}$		4.2	7.1		–	1.38		1.39	1.94	
$^*LSD_{5\%}$			10.0			1.95			2.74	

* Differences between treatments; Different letters within the columns indicate significant differences between the data pairs.

control and moderately large potassium ($K_{1.5}$) doses for both varieties. This means that a moderately high potassium level is advantageous for food quality, particularly in the case of drought.

Discussion

Increasing the plant density caused different changes in the yielding ability, thousand seed mass and susceptibility to *Xanthomonas phaseoli* in beans with various seed sizes. A rise in the plant density up to 400–450 thousand plants/ha had no effect on either yield or thousand seed mass in the small-seeded bean variety. Others authors (Velich and Unk, 1995) found that a plant density of 300–440 thousand plants/hectare was satisfactory for seed production in dry bean and French bean cultivars. Varieties such as Debreceni Gyöngy could take up fertilizer and water satisfactorily from a smaller growing space because of their slightly longer flowering period, small leaves and erect bush growth type, so they can be considered to be indifferent to a decrease of growing space and an increase in plant density. Varieties with determinate bush types and large leaves have larger nutrient and water requirements than bush beans with small leaves. With an increase in plant density, their growing space decreased leading to possible disturbances in the utilization of nutrients and water. Nemeskéri (2000) reported on the basis of the returns index that a density of 285–334 thousand plants/hectare was the most economical for producing beans with medium and large seeds. The largest returns index was achieved using good quality seed and the optimum seed norm (160–170 kg/ha), equivalent to 285–334 thousand plants/hectare, when producing large-seeded bean varieties. The thousand seed mass decreased when the Debreceni Tarka variety was grown at above optimum plant density (334 thousand plants/ha) even under favourable climatic conditions. The plant height of varieties with large leaves increased slightly at high plant density. These varieties need to have good stem stability because prostrate plants may produce a large proportion of diseased seeds, leading to a deterioration in seed quality. The results on the ratio of broken seeds were contradictory, as the ratio decreased at high plant density. The microclimatic conditions within a dense stand seemed to have a favourable effect on the seed moisture, resulting in a low ratio of broken seeds. Varieties susceptible to *Xanthomonas phaseoli* (such as Debreceni Tarka and Békési Fehér) produce a high ratio of diseased seeds, depending on the year, but this ratio did not increase significantly with a rise in plant density. The seed quality of these varieties was controlled by genetic factors rather than plant density.

There were differences between the bean varieties in the utilization of potassium. A high rate of potassium fertilizer ($K_{2.0}$ = 200 kg/ha) resulted in a decrease in the number of pods and seeds in the small-seeded bean variety Debreceni Gyöngy because of prolonged ripening. High potassium rates reduced the ratio of diseased seeds in this variety. Differences in protein content were demonstrated between the control and moderately large potassium doses, which was advantageous for food quality in the case of drought. There were no significant differences in the resistance or other quality factors of the large-seeded Debreceni Tarka variety, which is susceptible to bacterial diseases. According to the results, the variety Debreceni Gyöngy, which has small seeds and leaves, was able to utilize high levels of potassium better than the Debreceni Tarka variety, which has large seeds and leaves.

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YIELD AND YIELD COMPONENTS OF WHEAT SUBJECTED TO WATER STRESS UNDER RAINFED CONDITIONS IN PAKISTAN

R. H. KAZMI, M. Q. KHAN and M. K. ABBASI

UNIVERSITY OF AZAD JAMMU AND KASHMIR, RAWALAKOT, AJK-PAKISTAN

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The investigation was concerned with the effects of water stress on the yield and yield components of wheat grown under rainfed conditions in Rawalakot, Pakistan. A pot experiment was conducted with four wheat genotypes, Inqlab-91, Chakwal-97, Rawal-87 and Kohsar-95, tested against five irrigation levels with drought imposed at different growth stages including control, terminal drought, post-anthesis drought, three irrigations and pre-anthesis drought. The parameters studied were flag leaf area, ear stalk length, number of grains per spike and grain yield per pot. Flag leaf area and ear stalk length exhibited a significant reduction of 14 and 36%, respectively, when wheat was subjected to water stress. The proportional reduction in yield was 40% with three irrigations and 98% in the case of pre-anthesis drought depending upon the extent and degree of stress. Results showed that wheat could withstand and tolerate drought only up to anthesis, after which water stress resulted in the complete failure of the crop. It could be deduced that the critical stage for moisture in wheat started 60 days after germination, and became more severe at 90 days, i.e. at the anthesis stage. Among the genotypes, Inqlab-91 was found to be more tolerant of drought and could thus be a good option for further testing and recommendation for rainfed areas.

Key words: drought, genotypes, growth, irrigation, *Triticum aestivum* L.

Introduction

Drought is a serious problem that affects many regions of the world, decreasing the photosynthetic rate of crops and limiting productivity worldwide. It occurs when various combinations of the physical factors of the environment produce an internal water stress in crop plants sufficient to reduce their productivity (Larson, 1992). This reduction in productivity is brought about by a delay or prevention of crop establishment, weakening or destruction of established crops, predisposition of crops to insects and diseases, alteration of physiological and biochemical metabolism in plants, and alteration of the quality of the grain, forage, fibre, oil and other sought-for products (Larson and Eastin, 1971). Growth and photosynthesis are two of the most important processes disturbed, partially or completely, by water stress (Kramer and Boyer, 1995), and changes in both are a major cause of decreased crop yield. The worldwide losses in crop yield from water stress exceed the losses from all other losses combined (Kramer, 1980). Even a temporary drought can cause a substantial loss in crop yields, which can sometimes amount to many millions of dollars (Moseley, 1983). Therefore, water availability is an essential factor influencing agriculture. Water is generally considered as one of the limiting factors which affects the physiological and biochemical processes affecting crop productivity (Boyer, 1982).

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Reduced plant productivity due to drought is a major concern for wheat grown in arid and semiarid areas. In these areas, most wheat is grown under rainfed conditions where drought may occur at any time. About 37 % of the world's wheat is grown in semiarid areas where moisture is the most serious production constraint (Osmanzai et al., 1985). Rainfed areas play an important role in crop production in Pakistan. According to Ahmed et al. (1996) nearly one-fifth of the total wheat acreage in Pakistan is rainfed and contributes about 10-12% of the total wheat production in the country. The yield per hectare of rainfed wheat is discouragingly low, being 1130 kg ha^{-1}, about half that of irrigated wheat (Chaudhry et al., 2000). Among the different factors, drought (moisture stress) has emerged as a serious threat to productivity over the last 5–6 years when no rainfall has occurred during most of the year, especially during winter.

The best option for crop production, yield improvement and yield stability under soil moisture deficient conditions is to develop drought-tolerant crop varieties. A physiological approach would be the most attractive way to develop new varieties rapidly (Turner and Nicolas, 1987), but breeding for specific, suboptimal environments involves a deeper understanding of the yield-determining process (Blum, 1983). This is where knowledge of crop responses to water deficits may be best put to use. It has been claimed that breeding for drought tolerance can best be accomplished by selecting for grain yield under field conditions (Ashraf et al., 1996). Varietal differences in drought have been reported in wheat (Schonfeld et al., 1988), which could be exploited in appropriate breeding programmes. The screening of existing varieties for various physiological and yield parameters could be used to improve and select more tolerant varieties under specific conditions.

Genotype-environment interactions are of major importance in developing and identifying a cultivar performing well in diverse environments. The ranking and classification of cultivars according to their adaptability can be achieved through stability analysis, where high mean performance is the characteristic aimed at (Zubair et al., 2002). The release of genotypes with constant performance over a range of environments could lead to stability in production (Imdad et al., 1997). The aim of the present work was to study the effect of drought stress on the yield characteristics of wheat and to investigate drought-tolerant varieties to determine the most sensitive developmental stage of wheat plants to water stress.

Materials and methods

An experiment was conducted in the greenhouse, University College of Agriculture, Rawalakot, Azad Jammu and Kashmir (Pakistan) during 2000–2001, using plastic pots each measuring 450 cm^2 (22.5 cm × 20.0 cm) and 22 cm in depth. Each pot was filled with 10 kg soil collected from 0–15 cm depth. The surface soil was silty loam in texture, with pH 7.8, organic matter 0.90% and available P 2.5 mg kg^{-1}. After filling, the pots were levelled to ensure an even distribution of water. At field capacity level, a basal dose of nitrogen (90 kg N ha^{-1}) and phosphorus (60 kg P_2O_5 ha^{-1}) in the form of urea and SSP, respectively, were incorporated well into the soil. Four locally recommended winter wheat genotypes, namely Inqlab-91, Chakwal-97, Rawal-87 and Kohsar-95, were chosen for the study on the basis of their drought resistance. The following stress treatments were imposed to simulate the type of drought generally encountered in the region:

T_0 Control; four irrigations as recommended for wheat at tillering (30 days after sowing, DAS), initiation of heading (60 DAS), anthesis (90 DAS) and grain filling (120 DAS).

T_1 Terminal drought; only one irrigation at tillering (30 DAS)

T_2 Post-anthesis drought; two irrigations, one at tillering (30 DAS) and the second at the initiation of heading (60 DAS)

T_3 Three irrigations; at the initiation of heading (60 DAS), anthesis (90 DAS) and grain filling (120 DAS)

T_4 Pre-anthesis drought; two irrigations, at anthesis (90 DAS) and grain filling (120 DAS).

There were four genotypes, five irrigation levels and three replications, giving a total of 60 pots. The seeds of the four genotypes were obtained from the Crop Science Section, National Agriculture Research Center (NARC), Islamabad, Pakistan. Ten healthy seeds of each genotype were sown on October 25, 2000. After germination, thinning was done at the four-leaf stage and only four healthy plants were selected and maintained in each pot. Data were recorded for flag leaf area, ear stalk length, number of grains per spike and grain weight per pot according to standardized methods.

The data and means from each treatment were used for statistical analysis. Independent ANOVA was carried out for each parameter and least significant differences (LSD) were calculated at the 5% level of probability (Steel and Torrie, 1980). Traits showing significant genotype × irrigation stress interactions were subjected to stability analysis as proposed by Eberhart and Russell (1966). This model provides a means of partitioning the genotype × environment interaction of each genotype into two parts: (i) the variation due to the response of the variety to varying environmental index (sums of squares due to regression); and (ii) the unexplainable deviations from the regression on the environmental index.

Results and discussion

Wheat plants subjected to terminal drought (T_1) and post-anthesis drought (T_2) were severely damaged, could not recover and died in the 2nd week of March, so the present paper included the data of three water stress levels, i.e. the control (T_0), three irrigations (T_3) and pre-anthesis drought (T_4). In the present study, the critical stage of water for wheat was the later stages of plant development, i.e. the terminal and post-anthesis stages. Therefore, the absence or shortage of moisture during these stages in the T_1 and T_2 treatments under limited growth conditions (pots) did not allow the plants to continue their growth towards the productive (yield) stage. Moreover, continuous drought and the shortage of water changed the behaviour and characteristics of the soil, making it very hard and compact. Generally, Rawalakot soils are silt dominant, showing hard plasticity characteristics on drying. In an experiment on drought stress in wheat, Dhanda and Sethi (2002) reported that plant wilting commenced at 10–13% moisture level in the upper 15 cm layer of soil at anthesis and at 9–11% moisture at crop maturity. The moisture level of the soil was not measured, but it is believed that the moisture level in T_1 and T_2 may have been as low as 5–10%

only. However, it should be mentioned here that under similar experimental conditions, Ashraf (1998) reported wheat growth and yield even in terminal and pre-anthesis drought. The difference could be due to the condition and nature of the soil. Larson (1992) also reported that soil characteristics played an important role in the development of a critical range of water stress for a particular crop.

Since water plays an important role in plants, it is not surprising that reduced water absorption and dehydration have a deleterious effect on most physiological processes in the wheat crop. In the present study, the analysis of variance for the flag leaf area of four wheat genotypes across three water stress levels revealed a significant ($P \leq 0.01$) difference between the water levels and genotypes, while the interaction (genotype × stress) showed a non-significant difference. The flag leaf area of wheat plants exhibited a significant reduction under drought (Fig. 1). Among the three stresses developed, the maximum reduction of 18% was observed in pre-anthesis drought (T_4), while T_0 and T_3 exhibited almost similar response. Water provides turgidity to the cell while water stress causes dehydration, reducing the enlargement and expansion of the cell, resulting in a reduction in leaf area. The reduction in leaf area certainly affects the overall growth of the crop, because the greater the flag leaf area of a particular plant, the more will be the photosynthesis (Swati et al., 1985) and the greater will be the potential for growth and grain yield (Asana, 1968; Berdahl et al., 1972). The statistical analysis depicted significant differences between the varieties, indicating genetic differences between the wheat genotypes. Inqlab-91 had the maximum flag leaf area of 23.55 cm^2, while Kohsar-95 was severally damaged and had the lowest leaf area of 11.86 cm^2. The analysis showed a non-significant genotype × irrigation interaction, so the data could not be subjected to stability analysis.

Water stress imposed to wheat significantly reduced the ear stalk length, which provides the photosynthetic area vital for photosynthesis and affects the manufacture of plant food, and thus yield (Asana, 1968). Stability analysis for ear stalk length revealed highly significant differences between the water stress levels, genotypes and their interaction, while pooled deviation was also highly significant (Table 1). The maximum ear stalk length of 14 cm was recorded under normal irrigation, whereas the minimum length of about 9 cm was found in post-anthesis drought. Among the genotypes, Inqlab-91 had the maximum ear stalk length, while Kohsar-95 had the minimum. The differences between the genotypes may be due to their diverse germplasm, while the fluctuation in the ear stalk length under various water stress treatments may be due to a reduction in vegetative growth, which affected the length of the ear stalk. Swati et al. (1985) reported that the elongation of the ear stalk occurred at a later stage than that of other internodes and was thus affected by later drought. The stability analysis of ear stalk length for wheat genotypes evaluated across three water stress levels indicated highly significant differences and revealed genetic

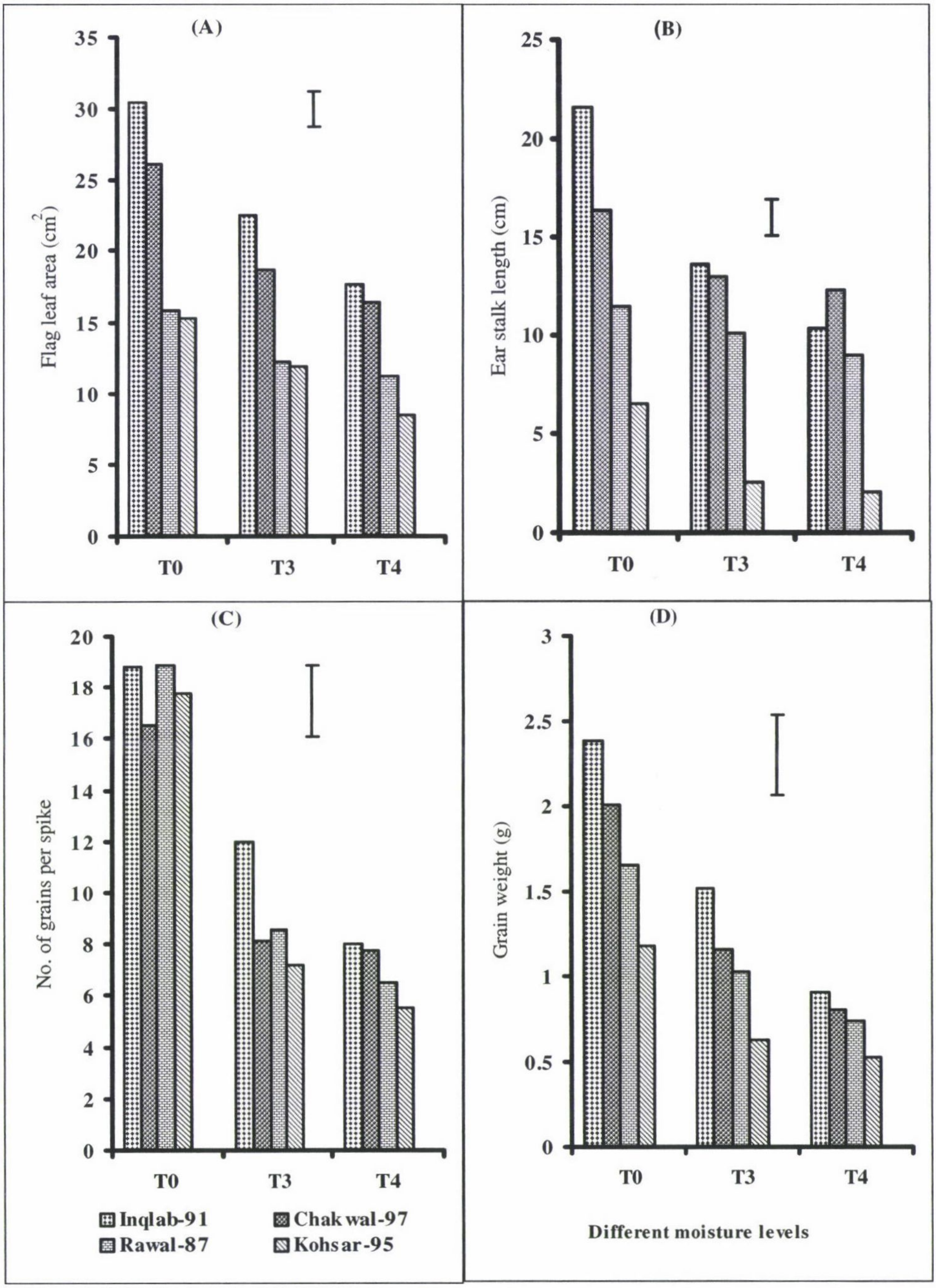

Fig. 1. Effect of different levels of water stress on (A) flag leaf area, (B) ear stalk length, (C) number of grains per spike and (D) grain weight of four genotypes of wheat. Vertical line bar indicates LSD value (P<0.05) between different stress levels

Table 1

Stability analysis of variance for ear stalk length (cm) for four wheat genotypes evaluated across different water stress conditions

S. O. V.	D. F.	S. S.	M. S.	F-ratio
Genotypes (G)	3	238.442	79.480	84.105**
Treatments (T)+(G × T)	8	91.608	11.451	
Treatments (Linear)	1	64.008	64.008	
G × T (Linear)	3	23.727	7.909	8.369**
Pooled deviations	4	3.781	0.945	0.863
Inqlab-91	3	2.512	0.837	0.764
Chakwal-97	3	0.509	0.169	0.154
Rawal-87	3	0.855	0.285	0.260
Kohsar-95	3	0.005	1.666	1.521
Pooled error	24	26.28	1.095	

*Significant at 5%, **Significant at 1%

Mean ear stalk length (cm) and estimate of stability parameters

Sr. No	Genotypes	X	b	S^2d
1	Inqlab 91	15.19	2.005	1.05
2	Chakwal 97	13.89	0.754	–0.953
3	Rawal 87	10.19	0.375	–0.607
4	Kohsar 95	3.70	0.869	–1.467

differences between the genotypes tested. The F-test for genotype × treatment (linear) was also highly significant, indicating genetic differences between the wheat genotypes for their regression on water stress. Likewise, the F-test for pooled deviations expressed highly significant differences. The regression coefficient for these genotypes ranged from 0.375 to 2.005. Two genotypes, Rawal-87 and Kohsar-95, had b values close to unity and were found to be stable when evaluated across the water stress levels. They had high mean values, with mean ‘b’ close to unity and S^2d close to zero.

The analysis of variance for number of grains per spike showed a significant difference ($P \leq 0.01$) between water stress levels, while genotypes and interaction (genotype x water stress) were non-significant. The number of grains per spike was reduced significantly by water stress depending upon the stage of drought (Fig. 1). A 50 to 60% reduction was recorded in T_3 and T_4, relative to the control where all the normal irrigations were applied. Ashraf (1998) reported post-anthesis drought as being the most sensitive stage for number of grains per spike in wheat. In the present study, plants grown under terminal and post-anthesis drought did not survive, so the data could not be correlated. However, the 50–60% reduction at the pre-anthesis stage was close to the findings of Ashraf (1998). Drought stress at anthesis and fertilization reduced the number of grains because of the dehydration of the pollen grains. Moreover, pollen grain germination and pollen tube growth down the style into the ovary and ovule were badly affected (Larson, 1992). The present findings were also supported by Hussain et al. (1987), Ashraf et al. (1989) and Qadir et al. (1999), who all reported a reduction in the number of grains per spike when the wheat crop was exposed to water stress. Among the genotypes tested, Inqlab-91 had the maximum number of grains.

Water stress caused a severe reduction in the grain yield of all four wheat genotypes tested (Fig. 1). The reduction was 40% in the three irrigations treatment and 98% in pre-anthesis drought. The sensitivity of the grain yield to drought stress depends upon the severity of the stress and the stage when it is imposed. Ashraf (1998) and Swati et al. (1985) reported a substantial grain yield of wheat when the stress was imposed in the post-anthesis stage. In the present work, a 98% reduction in grain yield was recorded even in the case of pre-anthesis drought, indicating the necessity of water in all critical stages of plant development. Drought stress has been shown to reduce translocation from the leaves, and as drought hastens maturation, this response, in addition to reducing photosynthesis, contributes to lower grain yield (Larson, 1992). In addition, shortage of assimilates and sometimes of nitrogen availability is a major cause of arrested grain and fruit growth during drought stress (Barraclough et al., 1989). It was also observed that water stress shrivelled the grains and that the degree of shrivelledness depended on the genotype. The shrivelledness of the grains affected the weight and ultimately the yield of the crop. Shrivelled wheat grain as a result of water stress was also reported by Ashraf (1998). Genotypes also showed a significant difference in yield potential. On average, Inqlab-91 had the maximum grain weight of 1.60 g pot^{-1}, while Kohsar-95 had the minimum of 0.77 g pot^{-1}, indicating genetic variation between the genotypes. The analysis showed a non-significant genotype×irrigation interaction, so the data could not be subjected to stability analysis.

The results obtained in the present investigation indicated that drought stress under Rawalakot conditions substantially decreased the yield and yield components of wheat. Wheat plants could withstand and tolerate drought if it occurred before anthesis, but water stress imposed in the later stages of plant development severely damaged the crop, resulting in the complete failure of the crop. It could be deduced that the critical period for moisture in wheat began 60 days after germination and became more severe at 90 days, i.e. at the anthesis stage. At this stage water shortage severely damaged the reproductive cycle and ultimately the yield of the crop. Under water stress, the hard, compact nature of Rawalakot soils makes the environment worse for crop growth and yield. However, Rawalakot generally receives a considerable amount of rainfall during the later part of winter from March onwards. This situation is very encouraging for wheat growers, as the critical stage of water for wheat proved to be the later stage of development. Wheat can thus be successfully grown under rainfed conditions in Rawalakot. Inqlab-91 showed superiority among the genotypes tested, but Chakwal-97 and Rawal-87 also exhibited characteristics of tolerance against drought. These genotypes may thus be a good option for further testing and recommendation for rainfed areas. The exploitation of these genotypes in breeding programmes for rainfed areas is highly recommended. The work reported in this paper has relevance to rainfed agriculture and may assist subsequent research in the screening and development of wheat varieties that are more resistant to drought conditions and thus help to improve crop yield in rainfed areas of the country.

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THE EFFECT OF NPK MINERAL FERTILIZATION ON THE ALVEOGRAPHIC PARAMETERS OF WINTER WHEAT

Z. GYŐRI, S. SZILÁGYI and P. SIPOS

INSTITUTE OF FOOD PROCESSING AND QUALITY ASSURANCE, CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, FACULTY OF AGRONOMY, DEBRECEN, HUNGARY

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Investigations on the baking quality of winter wheat, which is the most important bread cereal in Hungary, have been in the focus of attention for a long time. It is useful to study the theoretical and practical aspects of European quality testing systems, because different European methods are generally mutually accepted in the EU. Many recognised testing methods have been developed in Hungary over the last hundred years.

In the present experiments studies were made on the effect of the year, variety and mineral fertilization and their interactions on alveographic parameters. In the years examined, the main factor which determined the alveographic values was found to be the variety. Fertilization had a significant effect on the examined parameters, but in most cases no regular trends were observed. The year only modified wheat quality in interactions.

Key words: mineral fertilization, winter wheat varieties, quality, alveograph, technological parameters

Introduction

The quality of winter wheat can be examined by different methods based on the measurement of the rheological characteristics of dough made from wheat flour (farinograph, mixograph and alveograph). In Hungary the farinograph has been dominant up till now, but there is increasing interest in the alveograph due to the changing market and to EU requirements (Bartolucci et al., 1998; Dubois, 1996; Rasper et al., 1986). The questions which must be asked when a new method is introduced are:

- how can the data be explained?
- what kind of effects can be demonstrated using the new data?
- is it possible to compare the new data with others gained earlier with different methods? (Véha and Markovics, 1998; Fehér and Bányász, 1993; Matuz et al., 1981; Vida et al., 1996).

Benedek and Győri (1995) found a connection between alveographic values and cropping sites. Matuz et al. (1999) examined the effect of the year on these parameters and found that the year had a significant effect on the L, P/L and G values. Szilágyi (2000) examined the effect of fertilization on alveographical values in different years, but the results did not reveal unambiguously construable trends.

In order to find answers to these questions, the P, L, P/L, G and W values of the Chopin *Alveograph* were determined for other quality parameters. The main objective was to determine the effect of the year, variety and fertilization and their interactions on the alveographic parameters of the winter wheat varieties GK Öthalom, Fatima, Mv Magdaléna and GK Véka in the years 1998 and 1999.

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Materials and methods

In the fertilizer treatments there was a constant ratio of 1 N : 0.75 P_2O_5 : 0.88 $K_2O_{.5}$. The basic total dosage was 79 kg/ha, including 30 kg/ha N, and 1-2-3-4-5-fold amounts of the basic dosage were used with a non-fertilized control. Since all the treatments used the same NPK ratio, the dosages are referred to in the evaluation simply by the N content. The trial was set up in a strip arrangement with four replications. There were 6 random fertilizer treatments in each replication. The size of each fertilization plot was 21 m^2.

Soil characteristics. The soil of the Experimental Farm is calcareous chernozem with a basic layer of lowland loess. It has average N and P supplies and a high K supply (humus content 2.8–3%, total N 0.14–0.18%, AL-P_2O_5 130–200 mg/kg, AL-K_2O 240–280 mg/kg). The depth of the humus layer is 70–90 cm. The pH value (KCl) is 6.2.

The examined samples were taken from the Látókép Experimental Station of the Centre for Agricultural Science in the years 1998 and 1999. There was similar weather in the two years. The main difference was that in 1999 there was 70 mm precipitation in the week before harvest, while in 1998 this was only 17 mm Table 1. The varieties GK Öthalom, Fatima, Mv Magdaléna and GK Véka were investigated.

The quality parameters were determined in the Department of Food Science and Quality Assurance. Flour was made from winter wheat using a laboratory mill (type QC-106). The alveograph testing was conducted using a Chopin instrument with the AACC (1983) method. The effects of different years and agronomic factors were examined by analysis of variance (Sváb, 1973). The mean values and standard deviations of the examined parameters were represented in figures for each treatment.

Table 1

Precipitation figures for the experimantal years (Látókép, 1998–1999)

	Cropping year 1998	Cropping year 1999
October	9.70	42.60
November	37.50	59.30
December	58.30	24.90
January	17.90	9.00
February	1.20	60.10
March	8.20	18.60
April	87.70	68.00
May	85.80	53.80
1-10 June	28.20	10.50
11-20 June	32.70	79.60
21-30 June	17.60	27.50
1-10 July	57.10	6.00
10 July - harvest	17.60	70.30

Results and discussion

The results of the analysis of variance are shown in Table 2.

The *alveographic P value* showed a significant correlation with the variety, fertilization and their interaction. The effect of the year was not significant, but the year–variety interaction was. The other effects were not verified statistically.

Table 2
Analysis of variance of the experiment (MSQ values), Látókép, 1998–1999

Factor	P value	L value	P/L value	G value	W value
Year (a)	3642.00	161.33	0.263	3.75	113055.00
Error a	44.09	401.85	0.031	5.23	1924.27
Variety (b)	2350.40***	9863.38***	1.269***	129.73***	63259.95***
Year × Variety	2367.79***	3003.51***	0.153**	35.68**	33185.07**
Error b	58.82	144.86	0.0207	1.93	1068.36
Fertilization (c)	354.95**	944.07***	0.3839**	14.28***	750.43
Year × Fertilization	71.49	40.37	0.0290	0.67	377.35
Variety × Fertilization	174.87*	199.56	0.1003**	3.22	1337.81*
Year × Variety × Fertilization	134.78	278.72*	0.1102**	4.11**	975.03
Error c	83.33	139.28	0.0290	1.87	711.50

The alveographic P values of the tested varieties did not show the same tendencies for all fertilizer levels in the examined years. In 1998 GK Véka had the highest P value and Mv Magdaléna the lowest. Fatima showed a higher P value than GK Öthalom for the control plot and the first treatment, while the two varieties had similar P values at 60–90 kg/ha N. At the highest fertilizer levels GK Öthalom had the higher P value. In 1999 higher values were recorded. Fatima had the highest value (more than 80 mm), followed by Mv Magdaléna, GK Véka and finally GK Öthalom. Samples from the control plots did not exhibit this order (Fig. 1).

The analysis of variance showed very similar results for the alveographic L values. This parameter showed a significant correlation with the variety, fertilization, year-variety interaction and year-variety-fertilization interaction.

In 1998 the order of L values for the examined varieties was the same for all fertilization levels except the control. GK Öthalom had the highest value, followed by Fatima, GK Véka and Mv Magdaléna. In the control plot GK Véka had a higher value than Fatima. In 1999 the situation was different; no specific order could be established and the response of the varieties to fertilizer application was also different (Fig. 2*)*.

The alveographic P/L values showed a significant correlation with the variety and fertilization and also with the year–variety, variety–fertilization and year–variety–fertilization interactions.

In 1998 the variety Fatima had the highest P/L value without fertilization. In the other treatments the highest P/L value was shown by GK Véka, followed by Mv Magdaléna, Fatima and GK Öthalom. In 1999 a different order was found, which became more or less stabilized at the 60 kg/ha N+PK fertilization level. Mv Magdaléna and Fatima had the highest values. Summarizing the results of the P/L values, no regularity could be determined either for fertilizer application or for the varieties. The variety GK Öthalom exhibited more stable P/L values, while in the case of GK Véka the fluctuations were more pronounced (Fig. 3).

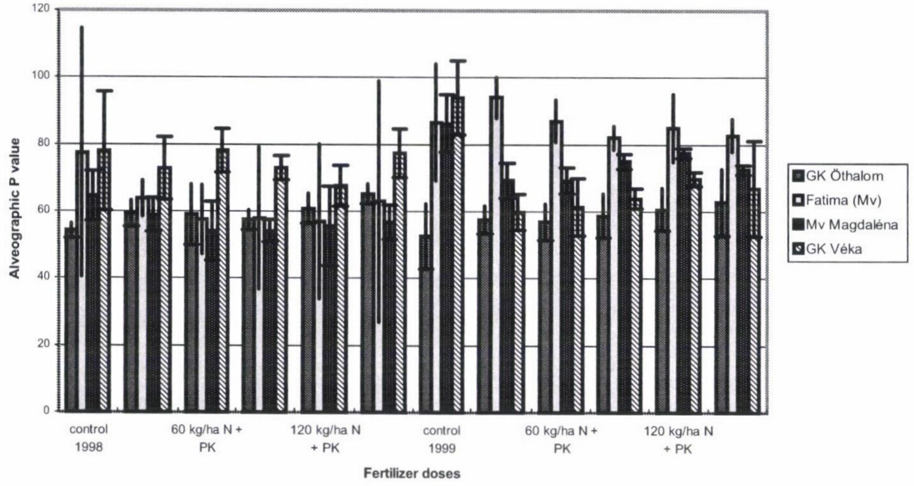

Fig. 1. Effect of fertilization on the alveographic P value of winter wheat varieties (Látókép, Hungary, 1998–1999)

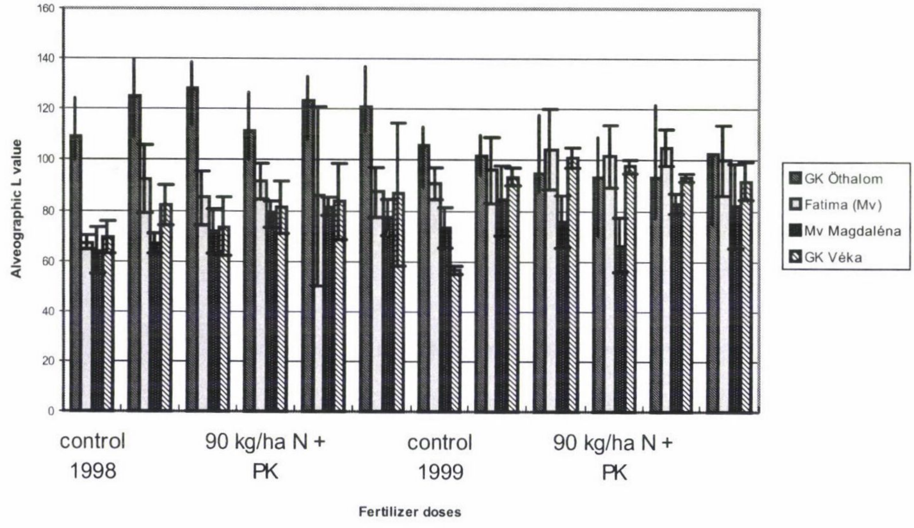

Fig. 2. Effect of fertilization on the alveographic L value of winter wheat varieties (Látókép, Hungary, 1998–1999)

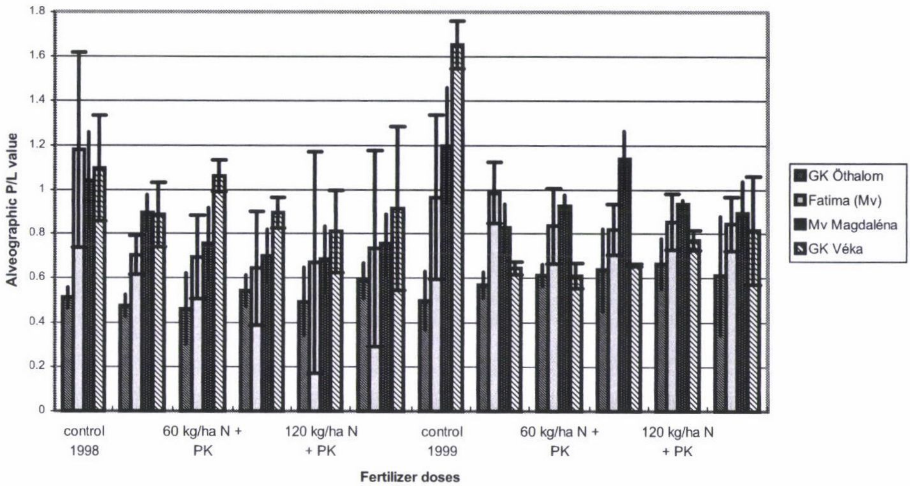

Fig. 3. Effect of fertilization on the alveographic P/L value of winter wheat varieties (Látókép, Hungary, 1998–1999)

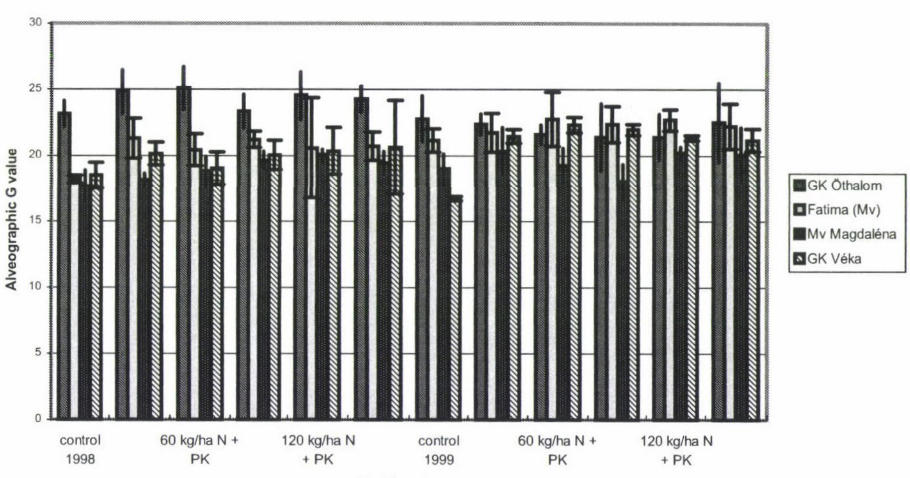

Fig. 4. Effect of fertilization on the alveographic G value of winter wheat varieties (Látókép, Hungary, 1998–1999)

Similar significant effects of the examined factors were observed for the alveographic G value as for the alveographic L value. GK Öthalom showed higher values in 1998 than in 1999, while there were no notable differences in the yearly performances of other varieties. In 1998 the order of varieties was the same as for the L value. In 1999 GK Véka and Mv Magdaléna had lower values in the control and Mv Magdaléna exhibited values lower than expected for several levels of fertilization as well (Fig. 4). Among the winter wheat varieties, GK Öthalom had the highest alveographic G values at every level of fertilization in 1998 and GK Öthalom and Fatima in 1999, while Mv Magdaléna had the lowest values except in the control treatment in 1999.

The alveographic W values showed a significant correlation with the variety, the year-variety interaction and the variety-fertilization interaction. The W value of the variety Mv Magdaléna was relatively low in both years examined (*Fig. 5*). The alveographic W value of the variety GK Öthalom did not show a definite direction of change as the result of mineral fertilization. This variety showed the highest values in 1998 and Fatima in 1999. However, Fatima showed the maximum W values in the N 30+PK treatment in 1999 and GK Öthalom in the N 60+PK treatment in 1998. Increasing levels of mineral fertilization did not bring about clear changes in the alveographic W value in the years examined.

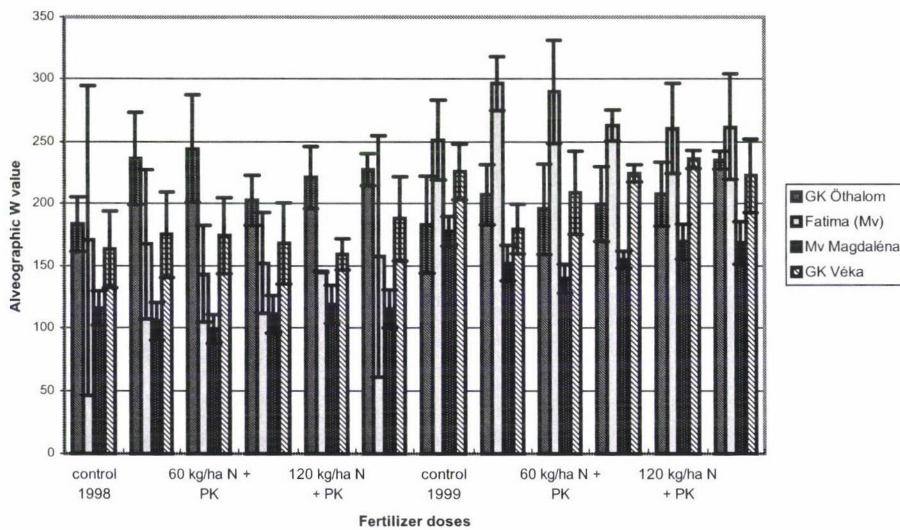

Fig. 5. Effect of fertilization on the alveographic W value of winter wheat varieties (Látókép, Hungary, 1998–1999)

Conclusions

All the alveographic parameters were found to be strongly dependent on the variety.

A year effect on the alveographic parameters was only observed in interactions. The effect of the variety on the examined parameters was much stronger.

The experiments did not demonstrate an unambiguous change in the alveographic parameters as the result of fertilization, though the effect was statistically significant.

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DEPENDENCE OF SEED MAIZE YIELD ON INTER-ROW SPACING AND SOWING DENSITY

P. JOVIN, L. ĐUKANOVIĆ, Ž. JOVANOVIĆ, M. VESKOVIĆ and J. TOŠIĆ

MAIZE RESEARCH INSTITUTE, ZEMUN POLJE, BELGRADE-ZEMUN, YUGOSLAVIA

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The seed maize hybrid ZP 677 was tested under irrigated conditions on the DPP Maglić estate in Bački Maglić during 2000 and 2001.

Sowing the female component of the hybrid ZP 677 at an inter-row spacing of 70 cm resulted in both higher yield (4.55 t ha^{-1}) and higher number of seeds per unit area (15,407,000) in comparison to sowing at an inter-row spacing of 35 cm, where the corresponding values were 4.39 t ha^{-1} and 14,667,000, respectively. At sowing densities of 71,425, 85,538 and 99,899 plants ha^{-1}, yields of 4.46, 4.38 and 4.59 th ha^{-1} were recorded, while the number of seeds per unit area amounted to 14,670,000, 14,769,000 and 15,686,000, respectively. The 1000-seed weight decreased on average for all seed fractions at both inter-row spacings as the sowing density increased.

Neither inter-row spacing nor sowing density affected the seed germination obtained with the standard test method, but germination in the cold test exhibited an insignificant increase with an increase in sowing density.

Key words: seed maize, yield, inter-row spacing, sowing density

Introduction

Maize seed production is one of the most profitable activities in agriculture providing that high yields are achieved per unit area. Some maize seed producers manage to achieve stable high yields within a standardised production. Among the many factors affecting the level of maize seed yield the following are the most important: soil and climatic conditions, the genotype of the female component, the effectiveness of pollination, and the level of cropping practices. The latter encompasses inter-row spacing and the sowing density of the female component, the effects of which were observed in the present study.

Several researchers (Ćirović, 1986; Jovin and Vesković, 1997; Jovin et al., 1999; Marinković and Starčević, 1989; Selaković, 1999), have studied the effects of inter-row spacing and sowing density on seed maize yields in Yugoslavia.

High yields in maize production are achieved by the use of hybrid seed of high quality. Seed is of the highest quality when the maximum dry matter weight is obtained, i.e. when it is physiologically mature. Good seed quality is demonstrated by its vigour, because seed with higher viability is more resistant to adverse agroecological conditions.

The aim of the present study was to determine the optimum sowing density for female components and to determine the inter-row spacing that would provide high seed yields while satisfying other necessary conditions.

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Materials and methods

The seed maize hybrid ZP 677 was tested under irrigated conditions on calcareous chernozem with good fertility on the DPP Maglić estate in Bački Maglić during 2000 and 2001. According to soil analyses (Živković et al., 1972), the pH of the ploughed layer amounted to 8.10 and 7.30 in H_2O and KCl, respectively. The contents of humus, total nitrogen, phosphorus and potassium amounted to 3.75–4.00%, 0.17%, 11.5 and 17.0 mg per 100 g of soil, respectively.

Mechanical sowing was carried out on optimum dates (April 29 and 30, 2000 and April 26, 2001) at inter-row spacings of 35 and 70 cm at densities higher than required. Upon completion of seed emergence, in the 5-6-leaf stage, the female components were manually thinned to the required densities (D_1: 71,425, D_2: 83,538 and D_3: 99,895 plants ha^{-1}). Since the forecrop in both years was potato, for which high rates of mineral fertilisers were applied, the soil for seed maize was fertilised with 300 kg $N_{15}P_{15}K_{15}$ ha^{-1} and 200 kg urea ha^{-1}.

Weed control and detasselling were performed as in all other seed maize plots. The trial was harvested on October 6, 2000 and September 30, 2001 with the simultaneous measurement of yield on plots from which ear samples were taken for the determination of grain moisture and cob weight. Upon drying and shelling the kernels were separated using a laboratory grader into four fractions (SF - small flat, SR - small round, LF - large flat and LR - large round). The 1000-seed weight was measured for each fraction.

The trial was set up with three replications and a 1: 3 ratio of female to male components. The plot size was 60.06 m^2.

The 1000-seed weight and total germination were tested in the laboratory under standard unfavourable conditions (cold test). The analyses were done in four replications except the tests of 1000-seed weight which were done in eight replications. The germination test was performed with a standard method on filter paper in a germination cabinet with alternating temperatures of 20 and 30°C and relative air humidity of 85% (Ujević and Kovaćević, 1972). The total germination was evaluated after seven days of testing (ISTA Rules, 1999). Degraded chernozem with a moisture content of 60% FWC was used for the cold test. The seed was exposed to unfavourable conditions (10°C) for seven days, followed by six days under optimum conditions (alternating temperature of 20 and 30°C).

The results were statistically processed using analysis of variance, while significant differences between the treatments were established by the LSD test.

Results and discussion

In both experimental years the seed maize yields obtained (Table 1) were lower at an inter-row spacing of 35 cm than at 75 cm, amounting to 4.54 and 4.64 t ha^{-1} (2000) and 4.25 and 4.48 t ha^{-1} (2001), respectively. The average yield of the female component over the two years was thus 4.39 and 4.55 t ha^{-1} at inter-row spacings of 35 and 70 cm, respectively. This difference in yield (0.16 t ha^{-1}) was not statistically significant.

Marinković and Starčević (1989) detected higher yields in the seed combination of the hybrid NSSC 75 at an inter-row spacing of 50 cm than at 60 or 70 cm. When studying the effects of inter-row spacing on the yield of the female component of hybrids ZPSC 704 and ZPSC 42A, Selaković (1999), recorded a higher yield at an inter-row spacing of 50 cm than at 70 cm.

An increase in the density led to a yield increase at an inter-row spacing of 35 cm averaged over both years (4.32, 4.34 and 4.52 t ha^{-1}). On the other hand, the yield varied over the densities at an inter-row spacing of 70 cm. The lowest yield (4.41 ha^{-1}) was achieved at the medium density, followed by the lowest (4.59 ha^{-1}) and the highest density (4.66 ha^{-1}).

Table 1
Effects of inter-row spacing and sowing density on yield (t ha^{-1})

Inter-row spacing (cm)	2000				2001				$\bar{x}$			
	Density											
	D_1	D_2	D_3	$\bar{x}$	D_1	D_2	D_3	$\bar{x}$	D_1	D_2	D_3	$\bar{x}$
35	4.38	4.55	4.70	4.54	4.26	4.14	4.35	4.25	4.32	4.34	4.52	4.39
70	4.70	4.51	4.70	4.64	4.48	4.33	4.64	4.48	4.59	4.41	4.66	4.55
$\bar{x}$	4.54	4.53	4.70	4.59	4.37	4.23	4.49	4.36	4.46	4.38	4.59	–

	for years	for inter-row spacing	for densities
F test	688^{*}	3.70^{NS}	2.19^{NS}
LSD 0.05	–	–	0.27
0.01	–	–	0.36

Averaged over the spacings, the yield obtained at the lowest and medium densities in 2000 was almost the same (4.54 vs. 4.53 t ha^{-1}), while the yield obtained at the highest density was higher (4.70 t ha^{-1}). In 2001, the yield obtained at the lowest, medium and highest densities was 4.37 t ha^{-1}, 4.23 t ha^{-1} and 4.49 t ha^{-1}, respectively.

The seed yield did not vary significantly over densities when averaged over years or inter-row spacings. The lowest yield (4.38 t ha^{-1}) was obtained at the medium density, followed by 4.46 t ha^{-1} and 4.59 t ha^{-1} at the lowest and highest densities, respectively.

When studying the effects of crop densities on the yield of the female component of the hybrid ZP 196, Jovin and Vesković (1997) reported higher yields at higher densities (71,400 and 85,700 plants ha^{-1}) than at a density of 57,100 plants ha^{-1}.

The 1000-seed weight was lower at an inter-row spacing of 70 cm than at 35 cm (Table 2). The average 1000-seed weight at an inter-row spacing of 70 cm amounted to 297.1 g and 295.0 g in 2000 and 2001, respectively, giving an average of 296.0 g over the two years. The corresponding values at an inter-row spacing of 35 cm were 301.2 g, 298.2 g and 299.7 g. The higher the density was, the lower the 1000-seed weight was, the values being 301.2, 295.6 and 291.2 g at an inter-row spacing of 70 cm, and 306.2, 297.8 and 295.1 g at an inter-row spacing of 35 cm.

Furthermore, a lower number of seeds (14,677,000 ha^{-1}) was found at an inter-row spacing of 35 cm than at 70 cm (15,407,000 ha^{-1}) (Table 3). In 2000 the higher the sowing density was, the higher the number of seeds (14,833,000, 15,229,000 and 16,00,000 ha^{-1}). In 2001, the lowest number (14,308,000 ha^{-1}) was recorded at the medium density and the highest number (15,373,000 ha^{-1}) at the highest density, while 14,508,000 seeds ha^{-1} were observed at the lowest density. On average, a higher number of seeds (14,670,000, 14,769,000 and 15,686,000 ha^{-1}) was recorded at higher densities for both years.

Table 2

1000-seed weight (g) as a function of inter-row spacing, sowing density and seed fraction

Inter-row spacing (cm)	Sowing density (plants ha^{-1})	Seed fractions	1000-seed weight (g)		
			2000	2001	$\overline{x}$
		SF	265.0	270.0	267.5
		SR	285.0	290.0	287.5
	71,425	LF	320.0	315.0	317.5
		LR	340.0	325.0	332.5
		$\overline{x}$	302.5	300.0	301.5
		SF	270.0	260.0	265.0
		SR	295.0	270.0	282.5
70	85,538	LF	300.0	320.0	310.0
		LR	320.0	330.0	325.0
		$\overline{x}$	296.2	295.0	295.6
		SF	270.0	255.0	262.5
		SR	280.0	270.0	275.0
	99,899	LF	300.0	310.0	305.0
		LR	320.0	325.0	322.5
		$\overline{x}$	292.5	290.0	291.2
Mean			297.1	295.0	296.0
		SF	275.0	270.0	272.5
		SR	300.0	295.0	297.5
	71,425	LF	315.0	310.0	312.5
		LR	350.0	335.0	342.5
		$\overline{x}$	310.0	302.5	306.2
		SF	270.0	270.0	270.0
		SR	285.0	280.0	282.5
35	85,538	LF	310.0	308.0	309.0
		LR	330.0	330.0	330.0
		$\overline{x}$	298.7	297.0	297.8
		SF	275.0	273.0	274.0
		SR	285.0	285.0	285.0
	99,899	LF	310.0	305.0	307.5
		LR	312.0	318.0	315.0
		$\overline{x}$	295.0	295.2	295.1
Mean			301.2	298.2	299.7

Jovin et al. (1999) achieved similar results for the yield of the female component of the hybrid ZP 677. These authors observed a higher number of germinated seeds at higher densities (85,538 and 99,895 plants ha^{-1}) than at a density of 71,425 plants ha^{-1}.

Germination using the standard test method was similar for both inter-row spacings and did not vary over densities in either of the experimental years.

Table 3

Effects of inter-row spacing and sowing density on the number of germinated seeds (1,000 ha^{-1})

Inter-row spacing (cm)	2000				2001				$\overline{x}$			
	Density											
	D_1	D_2	D_3	$\overline{x}$	D_1	D_2	D_3	$\overline{x}$	D_1	D_2	D_3	$\overline{x}$
35	14,129	15,233	15,932	15,098	14,083	13,939	14,746	14,256	14,106	14,586	15,339	14,677
70	15,537	15,226	16,068	15,610	14,933	14,678	16,000	15,204	15,235	14,952	16,034	15,407
$\overline{x}$	14,833	15,229	16,000	15,354	14,508	14,308	15,373	14,730	14,670	14,769	15,686	–

Table 4

Effects of inter-row spacing and sowing density on seed germination

Inter-row spacing (cm)	Sowing density (plants ha^{-1})	Germination (%)			
		2000		2001	
		Standard method	Cold test	Standard method	Cold test
	71,425	95	89	98	92
35	85,538	95	91	96	92
	99,895	95	92	97	94
70	71,425	95	90	94	88
	85,538	94	90	96	90
	99,895	95	92	98	93

The cold test is applied not only as a test comparative to the standard one, but also in order to evaluate the resistance of embryos and maize seedlings to unfavourable conditions during germination. The present study showed the real significance of the adequate, complete evaluation of seed viability. The values of seed germination obtained with this method were lower than those obtained with the standard method for both inter-row spacings and each density. Higher germination was detected in seed from higher sowing densities in both inter-row spacings (Table 4), but this difference was not significant.

Conclusions

The following conclusions can be drawn from the results.

The seed maize yields (4.55 and 4.39 t ha^{-1}) were uniform over the inter-row spacings (70 and 35 cm).

Compared with 71,425 plants ha^{-1}, the increase in the sowing density (85,538 and 99,895 plants ha^{-1}) did not significantly affect either the yield (4.38, 4.59 and 4.46 t ha^{-1}) or the number of germinated seeds (14,670,000, 14,769,000 and 15,686,000 ha^{-1}).

The 1000-seed weight decreased on average for all the seed fractions at both inter-row spacings as the sowing density increased.

Seed germination did not depend on either inter-row spacing or sowing density.

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VERTICAL DISTRIBUTION OF FORMS OF POTASSIUM IN MAJOR SOIL SERIES OF TAMIL NADU

S. NATARAJAN and A. RENUKADEVI

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Fifteen major soil series at the rate of three for each soil group, namely red non-calcareous, red calcareous, black calcareous, brown calcareous and alluvial soils, were studied for the vertical distribution of different forms of potassium. The total potassium content varied from 515–5513 ppm and generally increased with depth. It was positively related with clay, silt, $CaCO_3$, CEC and total Ca. Non-exchangeable K ranged from 340–1326 ppm and did not exhibit any uniformity in its distribution in soil profiles. The exchangeable K varied from 45–684 ppm and was positively related to organic carbon. NH_4OAc-K ranged from 15-298 ppm. Soil properties like clay, silt, $CaCO_3$ and organic matter were positively related to available K. Water-soluble K varied from 2–33 ppm and was found to decrease with the depth of the soil profile. It was positively correlated with organic carbon and negatively related to clay plus silt. Studies on the relationship between the above forms of potassium indicate that, except for total K, the other forms were closely related, indicating the possibility of predicting one from the other.

Key words: distribution pattern, forms of K, interrelationship, soil series

Introduction

A knowledge of potassium availability in soils is a pre-requisite when attempting to work out the response of crops to K in a given area. Potassium availability to plants is governed by the forms of K in the soils: water-soluble K, which is taken up directly by plants, exchangeable K, which is held by negative charges on clay particles and is available to plants, fixed K, which is trapped between layers of expanding lattice clays, and lattice K, which is an integral part of primary K-bearing minerals. Plants utilize not only the readily available K but also the less readily available K during their growth. The potassium availability to plants is determined by the rate of change in the dynamic equilibrium between different forms of potassium in the soil, which in turn is controlled by complex mineralogical factors, the rate of mineral weathering and the exchange properties of the soil (Tiwari and Bansal, 1992; Yadav et al., 1999). Hence, the present study was set up to determine the different forms of K in major soil series of Tamil Nadu.

Materials and methods

A total of fifteen major soil series at the rate of three for each soil group, namely red non-calcareous (Irugur: Igr, Vannapatti: Vpt and Vellalur: Vlr), red calcareous (Palladam: Plm, Tulukkanur: Tlk and Palathurai: Pth), black calcareous (Pilamedu: Plm, Dasarapatti: Dpt and Ammapettai: Amp), brown calcareous (Syamalakavundanpudur: Skp, Manuppatty: Mpv and Kuppandapalayam: Kpm) and alluvial soils (Koduveri: Kdv, Noyal: Nyl and Kallivalasu: Ksu) were chosen for the present study.

Fifty soil samples representing the genetic horizons of the profiles were collected and analysed for the various fractions of K. The key profile properties of the soils are presented in Table 1.

Table 1
Key profile characteristics of the soils

Profile No.	Soil Series	Depth in cm	Colour		Texture		Structural development in subsoil	Reaction to dil. HCl	Special features
			Surface	Sub-Surface	Surface	Sub-Surface			
1.	Igr	65	Yellowish red	Dark reddish brown	Sandy clay loam	Sandy clay loam	Moderate	Nil	Angular quartz gravels distinctly present just above the underlying weathered gneiss
2.	Vpt	40	Yellowish red	Dark red	Sand	Sandy clay loam	Moderate	Nil	Textural B horizon
3.	Vlr	85	Dark red	Red	Sandy clay loam	Sandy clay	Moderate	Nil	Iron gravels present at lower depth of profile
4.	Pld	28	Reddish brown	Reddish brown	(Gravelly) sandy loam	Loamy sand	Weak	Violent	$CaCO_3$ nodules in the C horizon
5.	Tlk	50	Yellowish red	Reddish brown	(Gravelly) sandy loam	Gravelly sandy loam	Moderate	Violent	Calcareous gneiss is the parent material
6.	Pth	50	Dark reddish grey	Dark reddish brown	Sandy loam	(Gravelly) sand clay loam	Moderate	Violent	$CaCO_3$ + gneiss (parent material)
7.	Plm	115	Very dark grey	Very dark grey to black		Clay	Moderate to strong	Violent	Distinct slicken-slides below 18 cm
8	Dpt	120+	Very dark greyish brown	Dark greyish brown and yellowish brown	Clay	Silty clay loam to clay	Strong	Violent	Distinct slicken-slides below 20 cm depth; gypsum nodules occur below 92 cm
9.	Amp	136+	Dark greyish brown	Very dark greyish brown	Sandy clay	Clay	Strong		Slicken-slides below 35 cm depth
10.	Skp	28	Yellowish brown	Strong brown	(Gravelly) loamy sand		Strong	Violent	$CaCO_3$ nodules as parent material
11.	Mpy	32	Dark yellowish brown	Brown to dark yellowish brown	Sandy clay loam	(Gravelly) sandy clay loam to sandy loam	Strong	Violent	Calcareous gneiss (parent material)
12.	Kpm	60	Dark brown	Brown to dark yellowish brown	Sandy clay loam	(Gravelly) sandy clay loam to sandy loam	Strong	Violent	Calcareous gneiss (parent material)
13.	Kdv	120+	Dark greyish brown	Very dark greyish brown	Sandy loam	Sandy clay	Strong	Slight	———
14.	Nyl	143+	Reddish brown	Reddish brown to dark	Sandy loam	Loamy sand to sandy clay loam	Weak	Nil	Stratification of layers within profile
15.	Ksu	110+	Very dark brown	Yellowish red to reddish brown	Sandy clay loam	Sandy loam to sandy clay	Strong	Nil	Stratification of layers within profile

Two grams of soil sample was boiled for 10 min with 20 ml 1N HNO_3 over a hot plate. After dilution and allowing to cool, the content was filtered into a 100 ml volumetric flask. The soil residue was washed thrice with dil. 0.1 N HNO_3 and the washings were also collected. After making up to the volume, non-exchangeable K was obtained by subtracting neutral 1 N NH_4Oac-soluble K from boiling 1 N HNO_3-soluble K and determined using an EEL flame photometer (Wood and De Turk, 1940).. For total potassium the soil was decomposed with a HF-$HClO_4$ mixture (Pratt, 1965). Exchangeable K was determined in the ammonium acetate leachate obtained during the cation exchange determination of the soils.

A 10 g soil sample was placed in a shaking bottle and 20 ml distilled water was added. The bottle was shaken in a mechanical shaker for two hours, left to stand overnight, and filtered. Water soluble K was determined from the filtrate, using an EEL flame photometer (MacLean, 1960). Five grams of soil was shaken with 25 ml of neutral normal NH_4OAc for 5 min and filtered for the determination of *N* NH_4OAc-soluble K (Hanway and Heidal, 1952).

Results and discussion

The different forms of K, namely total, non-exchangeable, exchangeable, ammonium acetate-soluble and water-soluble K, estimated in the horizons of profile samples of the fifteen soil series are presented in Table 2.

Total potassium

The values of total K as determined in the HCl extract ranged from 515 to 5513 ppm with a mean of 2371 ppm. The respective mean values for red non-calcareous, red calcareous, black calcareous, brown calcareous and alluvial soils were 2366, 2158, 3391, 1942 and 1966 ppm. The general trend showed an increase in total K with depth, but in the Kuppandapalayam series, total K decreased with depth. No uniformity in the distribution of K was noted in black calcareous and alluvial soils. Total K was not related with any other forms of K except NH_4OAc-K. It was positively related with clay, silt, clay plus silt, $CaCO_3$, total calcium and CEC. The total K content in the soil groups followed the order: black calcareous > red non-calcareous > red calcareous > alluvial > brown calcareous soils. The low total K in brown soils and in the Vannapatti series could be attributed to the intense weathering the soils had undergone, leading to a reduction in accessory minerals and to a coarse texture. Black soils, containing large quantities of clay and silt, had higher total K.

The total K generally increased with depth. Its distribution was a reflection of K fixation and correlated with the factors clay, silt, $CaCO_3$ and CEC (Kalbande and Swamynatha, 1976). Koria et al. (1989) reported that K-rich minerals in the subsurface layer associated with the finer fraction of the soils were responsible for the depth-wise increase in the total K content of the soil.

Table 2
Depth-wise distribution of different forms of potassium in soils

1	2	3	4	5	6	7	8
			(i) Red non-calcareous soils				
1.	Igr. 1	0–14	2318	649	320	227	14
	Igr. 2	14–40	2318	582	251	190	6
	Igr. 3	40–65	3710	610	128	79	4
2.	Vpt. 1	0–18	1000	360	65	40	10
	Vpt. 2	18–40	1030	340	45	20	6
3.	Vlr. 1	0–16	1785	362	95	97	33
	Vlr. 2	16–42	2600	364	76	52	2
	Vlr. 3	42–60	2860	454	102	68	2
	Vlr. 4	60–85	3675	583	180	100	4
	Mean		2366	478	134	97	9
			(ii) Red calcareous soils				
4.	Pld. 1	0–10	2040	1025	125	97	2
	Pld. 2	10–28	2273	944	99	66	2
5.	Tlk. 1	0–15	1020	474	149	87	4
	Tlk. 2	15–30	1010	480	99	25	2
	Tlk. 3	30–50	1030	345	49	15	2
6.	Pth. 1	0–15	2778	1318	464	298	16
	Pth. 2	15–38	3030	1177	684	237	8
	Pth. 3	38–50	4080	1326	223	204	6
	Mean		2158	886	223	129	5
			(iii) Black calcareous soils				
7.	Plm. 1	0–18	3710	742	236	265	2
	Plm. 2	18–50	3120	624	154	156	2
	Plm. 3	50–115	3478	647	132	155	2
8.	Dpt. 1	0–20	3090	427	199	242	6
	Dpt. 2	20–44	5200	796	178	244	4
	Dpt. 3	44–66	5513	798	180	252	4
	Dpt. 4	66–92	3900	759	180	229	2
	Dpt. 5	92–120	4200	819	180	231	4
9.	Amp. 1	0–10	1785	444	202	168	2
	Amp. 2	10–35	2080	458	180	166	2
	Amp. 3	35–90	2318	484	152	134	2
	Amp. 4	90–136	2295	398	125	112	2
	Mean		3391	616	174	196	3
			(iv) Brown calcareous soils				
10.	Skp. 1	0–28	1768	510	147	96	4
11.	Mpy. 1	0–20	2525	1076	316	288	12
	Mpy. 2	20–32	2778	1156	297	257	6
12.	Kpm. 1	0–15	2040	784	174	133	4
	Kpm. 2	15–40	1530	510	74	51	2
	Kpm. 3	40–60	1010	409	74	45	2
	Mean		1942	741	162	145	5
			(iv) Alluvial soils				
13.	Kdv. 1	0–12	1010	662	247	197	30
	Kdv. 2	12–33	2550	811	219	209	10
	Kdv. 3	33–63	2060	464	204	206	2
	Kdv. 4	63–97	2060	427	101	139	2
	Kdv. 5	97–120	1820	437	176	83	6

Table 2 continued

14.	Nyl.1	0–14	3060	1204	200	122	4
	Nyl. 2	14–33	2778	1035	198	126	4
	Nyl. 3	33–62	3315	1224	223	153	6
	Nyl. 4	62–115	3570	1326	151	82	2
	Nyl. 5	115–143	1515	672	99	35	2
15.	Ksu. 1	0–20	765	255	74	51	2
	Ksu. 2	20–41	765	281	49	25	2
	Ksu. 3	41–48	515	484	49	31	2
	Ksu. 4	48–88	2080	1076	154	98	2
	Ksu. 5	88–110	2080	1092	128	52	2
	Mean		1996	763	151	105	5
Overall mean			2371	697	169	134	6

1: Profile No.; 2: Soil series and horizons; 3: Depth (cm); 4: Total K (ppm); 5: Non-exchangeable K (ppm); 6: Exchangeable K (ppm); 7: Ammonium acetate-soluble K (ppm); 8: Water-soluble K (ppm)

Non-exchangeable potassium

The non-exchangeable K content varied from 340 to 1326 ppm with a mean of 697 ppm. This form of K represented 29.40% of the total K. Red calcareous soils registered a high content of non-exchangeable K (886 ppm), followed by alluvial soils (763 ppm), brown calcareous soils (741 ppm), black calcareous soils (616 ppm) and red non-calcareous soils (478 ppm). In general there was no uniformity in the distribution of non-exchangeable K within the profiles. A regular decrease in K with depth was observed in the Vannapatti, Palladam and Kuppandapalayam series, while an increasing trend was exhibited by the Manuppatty series. Non-exchangeable K was found to be related with exchangeable and NH_4OAc-K but was independent of the other soil properties. The non-exchangeable K content in the soil groups followed the order: red calcareous > alluvial > brown calcareous > black calcareous > red non-calcareous soils. It was low in the Vannapatti series (340 ppm) and high in the Palathurai series (1326 ppm). The difference in the intensity of weathering contributed to these variations.

The non-exchangeable K did not follow a regular distribution pattern in many of the soil series, as reflected in the absence of a correlation between non-exchangeable K and the soil properties which influenced K fixation or release.

Exchangeable potassium

The exchangeable K values ranged from 45 to 684 ppm with a mean of 169 ppm. This form of K constituted 7.12% of the total K. The mean values of exchangeable K content for red non-calcareous, red calcareous, black calcareous, brown calcareous and alluvial soils were 134, 223, 174, 162 and 151 ppm, respectively. Exchangeable potassium generally decreased with depth except in Vellalur, Palathurai and all the alluvial soil series. This K was found to be related with non-exchangeable, ammonium acetate-soluble and water-soluble forms of K. It was also positively correlated with organic carbon. The exchangeable K contents in the soil groups were in the following order: red calcareous > black calcareous > brown calcareous > alluvial > red non-calcareous soils. It varied from 45 ppm (Vannapatti series) to 684 ppm (Palathurai series). The nature and amount of clay and the presence or absence of K-bearing minerals influenced these variations.

Exchangeable K generally decreased with depth. This could be attributed to (i) the high degree of weathering of the surface soil, (ii) the release of soluble K from organic residues, (iii) the addition of K fertilizers to the ploughed zone, and (iv) the upward translocation of soluble K due to the capillary rise of the groundwater (Venkatesh and Satyanarayana, 1994).

The Vellalur series showed an increase in exchangeable K with depth, due to intensive elluviation and illuviation.

Ammonium acetate-soluble potassium

The NH_4OAc-K content ranged from 15 to 298 ppm with a mean of 134 ppm, and made up 5.66% of total K. The respective mean values for red non-calcareous, red calcareous, black calcareous, brown calcareous and alluvial soils were 97, 129, 196, 145 and 105 ppm. This form of K generally decreased with depth. The Vellalur, Dasarapatti and alluvial soil series showed variation within the profiles. NH_4OAc-K was significantly correlated with the total, non-exchangeable, exchangeable and water-soluble forms of K. It was also positively related with the clay, silt, clay plus silt, organic carbon and $CaCO_3$ contents of the soils. This form of K followed the order: alluvial > black calcareous > brown calcareous > red calcareous > red non-calcareous soils.

NH_4OAc-K decreased with depth as noted by Chandel et al. (1976). High organic matter and the addition of fertilizers in the ploughed zone increased the availability of potassium. The different behaviour of available K in the profiles of Vellalur and Dasarapatti and in alluvial soils was due to the stratification of the layers.

Water-soluble potassium

The water-soluble K content varied from 2 to 33 ppm with a mean of 6 ppm. This form of K constituted 3.55% of the exchangeable K and 0.25% of the total K. The mean values of water-soluble K for red non-calcareous, red calcareous, black calcareous, brown calcareous and alluvial soils were 9, 5, 3, 5 and 5 ppm, respectively. There was a general decrease in water-soluble K with depth, while in the Koduveri, Kallivalasu and Dasarapatti soil series no regular trend was noted with depth.

Water-soluble K was related to exchangeable and NH_4OAc-soluble K. This form of K was positively correlated with organic carbon while it was negatively related to clay plus silt. The water-soluble K content followed the order: red non-calcareous > red calcareous = brown calcareous = alluvial > black calcareous soils. It was low in the silt and clay fractions and hence black soils containing higher amounts of clay and silt showed low values.

Like exchangeable K, water-soluble K also decreased with depth. The addition of K fertilizers may have increased its content in the surface layers (Koria et al., 1989; Tiwari and Bansal, 1992). The stratification of the layers could be responsible for the alternating trend of water-soluble K in the profiles of Dasarapatti and alluvial soils.

Interrelationship among forms of potassium

Correlation coefficients were calculated to study the relationship between forms of K (Table 3). This revealed that the total K had no relationship with the non-exchangeable, exchangeable and water-soluble forms of K. This was possibly due to the intense weathering of the soils and the absence of K-bearing minerals. A correlation was observed between total and NH_4OAc-K, as reported by Bolan (1976).

The significant correlations obtained between non-exchangeable K and both exchangeable and NH_4OAc-K showed the existence of an equilibrium between these forms of K (Chahal et al., 1976). Exchangeable K, NH_4OAc-K and water-soluble K were found to be intercorrelated, indicating the existence of an equilibrium between them (Yadav et al., 1999).

The overall study on the correlations between forms of K revealed a reversible equilibrium between them, except for total K, which was not related to other forms. Hence the total K content is a poor guide of its availability to plants and even soils rich in total K might respond to K fertilization.

Relationship with soil properties

The relationship with different soil properties is presented in Table 4.

Any change in the soil system brings about an appropriate shift in the soil equilibrium. Various soil properties, such as clay, silt, $CaCO_3$ and organic matter determine the content of different forms of K. The clay and silt fractions were correlated significantly with total and NH_4OAc-K, as observed by Krishnamoorthy et al. (1976). The organic carbon content of the soil was related to water-soluble, exchangeable and NH_4OAc-K, which could be primarily due to the increased CEC and exchangeable K, and also due to the supply of K by the organic matter itself. Similar observations were made by Basumatary and Bordoloi (1992).

Table 3

Results of statistical analysis for inter-correlation between different forms of potassium (n=50)

No.	X Variable	Y Variable	Correlation coefficient (r)	Prediction equation (Y = a + bx)
1.	NH_4OAc-K	Total K	0.279*	Y = 107.37+24.22x
2.	Exchangeable K	Non-exchangeable K	0.567**	Y = 414.54+1.62x
3.	NH_4OAc-K	Non-exchangeable K	0.440**	Y = 467.92+1.68x
4.	Water-soluble K	Non-exchangeable K	0.734**	Y =39.59+0.55x
5.	Water-soluble K	Exchangeable K	0.307*	Y = 143.89+5.32x
6.	Water-soluble K	NH_4OAc-K	0.280*	Y = 114.81+3.64x

Table 4
Results of statistical analysis for correlation between soil properties (X) and forms of potassium (Y) (n=50)

No.	X Variable	Y Variable	Correlation coefficient (r)	Prediction equation (Y = a + bx)
1.	Clay	Total K	0.475**	Y = 1350.51+31.44x
2.	Clay	NH_4OAc-K	0.279*	Y = 93.15+1.28x
3.	Silt	Total K	0.388**	Y = 2043.39+47.13x
4.	Silt	NH_4OAc-K	0.297*	Y = 113.40+2.61x
5.	Clay + Silt	Total K	0.609**	Y = 1190.90+30.55x
6.	Clay + Silt	Water-soluble K	-0.296*	Y = 8.627-0.08x
7.	Clay + Silt	NH_4OAc-K	0.336**	Y = 85.18+1.22x
8.	Organic carbon	Exchangeable K	0.357*	Y = 93.10+232.97x
9.	Organic carbon	Water-soluble K	0.300*	Y = 1.46+11.31x
10.	Organic carbon	NH_4OAc-K	0.489**	Y = 52.94+239.28x
11.	$CaCO_3$	Total K	0.279*	Y = 1914.18+232.50x
12.	$CaCO_3$	NH_4OAc-K	0.429**	Y = 78.10+25.91x
13.	Total Ca	Total K	0.512**	Y = 1513.81+480.06x
14.	CEC	Total K	0.605**	Y = 1440.29+24.87x

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EFFECT OF METAL NON-ADAPTED ARBUSCULAR MYCORRHIZAL FUNGI ON Cd, Ni AND Zn UPTAKE BY RYEGRASS

T. TAKÁCS and I. VÖRÖS

RESEARCH INSTITUTE FOR SOIL SCIENCE AND AGRICULTURAL CHEMISTRY OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY

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The aim of the present study was to observe the effect of metal non-adapted arbuscular mycorrhizal fungi (AMF) application on the metal uptake of the host plant in a pot experiment. The soil samples originated from a calcareous chernozem soil treated with Cd, Ni and Zn with ryegrass (*Lolium perenne* L.) as the test plant. Changes in the parameters of mycorrhizal root colonization and in the metal uptake of the host as affected by metal type, metal rate and mycorrhizal treatments were investigated. Efficient mycorrhizal symbiosis was found to develop; the heavy metal uptake of the plants decreased in the presence of this symbiosis with metal non-adapted AMF.

Key words: ryegrass, heavy metals, arbuscular mycorrhizal fungi

Introduction

Arbuscular mycorrhizal fungi (AMF) are an abundant component of the soil biota in most terrestrial ecosystems (Harley and Harley, 1987). Their potential benefits to plant growth as obligatory mutualistic symbionts are widely recognized (Marschner and Dell, 1994). Mycorrhizae promote nutrient transfer from the soil to the roots of the host plant (Marschner, 1997). Plant-AMF symbiosis may alter the growth of the host plant and may play an important role by increasing the stress tolerance of the host-plant. Data on the heavy metal uptake of mycorrhizal plants and on the role of AMF in metal uptake and transfer in the host plant are contradictory (Leyval et al., 1997; Vörös et al., 1998).

High metal concentrations in the soil are toxic to soil microorganisms including AMF. Heavy metals have been reported to inhibit AMF sporulation (Díaz and Honrubia, 1993), AM spore germination and hyphal extension *in vitro* (Weissenhorn et al., 1993; 1994), and to reduce or completely eliminate the AM colonization of plant roots. Poor or absent mycorrhizal inocula were found in several mine spoils, which could explain the lack of mycorrhizal colonization. However, mycorrhizal plants were able to colonize polluted mining sites rather than non-host plants (Bothe et al., 1996; Shetty et al., 1994), suggesting that heavy metal tolerance or other beneficial effects were conferred by mycorrhizal symbiosis. The objective of this study was to determine the effects of 30, 90 and 270 mg kg^{-1} rates of Cd, Ni, and Zn treatments on the mycorrhizal colonization of ryegrass and the influence of AMF infection on the metal uptake of the host plant.

Materials and methods

Soil sampling and preparation

The soil originated from selected plots of a long-term field experiment at Nagyhörcsök (Hungary) and was classified as a calcareous loamy chernozem with the following characteristics: $pH_{(H_2O)}$: 7.5; $pH_{(KCl)}$: 7.2; $CaCO_3$ content: 5–6.5%; humus content: 3%; clay fraction: (< 0.002 mm) 20%; silt (0.02–0.05 mm): 40%. Inorganic salts (sulphates) of the trace elements Cd, Ni and Zn were mixed into the ploughed layer, each at 4 levels (0, 30, 90 and 270 mg metal/kg dry soil), on the experimental site in 1991 (Kádár, 1995). In the eighth year of this experiment bulk soil samples were taken from the upper 20 cm layer for a pot experiment. All soil samples were sterilised by gamma irradiation (with 25 kGy kg^{-1}).

The following treatments were set up:

1) Sterilised soil without AMF or other soil microrganisms (S);

2) sterilised soil re-inoculated with a non-adapted AMF population from unpolluted soil (10 w/w % AMF inoculum pot^{-1}) (S+AMF) and

3) sterilised soil re-inoculated with a mycorrhizae-free soil suspension (80 ml pot^{-1}) (S+B).

The AMF inoculum from the natural ecosystem contained potentially infective AMF propagules (AMF spores and AMF colonised roots). The soil suspension was prepared from 400 ml distilled water by filtering 0.2 kg^{-1} soil through a 5 μm pore size sieve to exclude AM fungi.

Plant growth

Perennial ryegrass (*Lolium perenne* L.) was grown in pots (with 180 g soil and 0.5 g seeds pot^{-1}, in three replicates) as the host of AMF for three months in a growth chamber under controlled climatic conditions (temperature between 25° and 17°C, with a 18/6 h light/dark period). The dry biomass development of the roots and shoots was measured, as well as plant metal concentrations and the parameters of mycorrhizal infection.

Chemical analyses

Available soil metal concentrations were determined in an extract using acid ammonium acetate + EDTA solvent (Lakanen and Erviö, 1971) (Table 1). The plant metalconcentrations were measured after wet digestion of the air-dried plant samples with HNO_3+H_2O_2 by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Mycorrhizal parameters and AMF spore extraction

The frequency (F%) of mycorrhizal infection and the quantity of arbuscules (a%) in the roots of the host were estimated by rating the density of infection on 30 cm root segments using the five class system (Trouvelot et al., 1986).

AMF resting spores were isolated from the untreated control soils and collected by wet sieving using the method of Gerdemann and Nicolson (1963). The intact, mature spores were selected under a binocular microscope and stored in polyvinyl-lacto-glycerol (PVLG) (Koske and Testier, 1983). The identification of isolated spores was made according to morphological properties.

Statistical analysis

All chemical data are given as the means of triplicate analyses of soil or plant samples. Standard errors of the means and least significant difference values (LSD, $P < 0.05$) were calculated and are represented as numerical values.

Results and discussion

The spores of 6 species of AMF (*Glomales, Zygomycota*) were isolated, described and illustrated from the natural ecosystem. Six AMF species were identified, namely *Glomus constrictum* (Trappe, 1977), *Glomus claroideum* (Skou and Jakobsen, 1989), *Glomus mosseae* (Nicolson and Gerdemann, 1968), *Glomus microcarpum* (Berch and Fortin, 1984), *Sclerocystis sinuosa* (Gerdemann and Bakshi, 1976), and another *Glomus* sp. from the metal non-polluted soils. The spores of *Sclerocystis sinuosa*, *Glomus constrictum* and *Glomus microcarpum* were also collected in three different habitats of semiarid open sandy grasslands in Hungary (Bratek and Takács, 1997). The *Glomus mosseae* and *Glomus claroideum* species were isolated from heavy metal polluted soil by several researchers (Del Val et al. 1999; Sambandan et al., 1992; Weissenhorn et al., 1993, 1994).

The Cd, Ni and Zn availability was high in the soil samples and reduced the plant biomass production (Tables 1–2). Among the various metal treatments all the Cd concentrations exceeded the permissible limit for soils several times. The shoot and root dry matter yields of ryegrass were slightly lower for mycorrhizal than for non-mycorrhizal plants (Table 2). However, no significant differences were generally recorded between mycorrhizal (S+AMF) and non-mycorrhizal (S or S+B) plants for either the root or shoot dry matter at high metal rates. Tonin et al. (2001) found that Cd-tolerant *Glomus mosseae* (BEG 69) reduced white clover growth in Cd-polluted soil and slightly increased the Cd concentration. Ruiz-Lozano and Azcon (2000) reported that *Glomus* sp.-colonized plants isolated from saline soils exhibited less growth and accumulated less N and P under salinity; however, *Glomus* sp. is known to protect plants from the detrimental effects of salt. A substantial proportion of the net photosynthates allocated to the roots is required for fungal growth and maintenance in mycorrhizal roots. In mycorrhizal plants "root" respiration may be 20–30% greater than in non-mycorrhizal plants, and 87% of the higher respiration of the mycorrhizal plants can be attributed to the fungus (Baas et al., 1989).

The Cd, Ni and Zn concentrations of different organs of ryegrass increased substantially when the metal levels in the soil were higher (Table 3). The Cd, Ni and Zn concentrations differed greatly in the roots and shoots. Metal accumulation was greater in the roots than in the shoots in all the metal treatments and in both mycorrhizal and non-mycorrhizal plants. The highest concentrations in plant tissues were found for Zn and the lowest for Cd. Possibly, interactions between the fungi and the host plant provide selective transport mechanisms between essential and toxic metals. In the presence of AM fungi the uptake of all three metals by the plant was significantly affected (Table 3). The metal concentrations both in the roots and shoots of mycorrhizal ryegrass (S+AMF) were lower than in non-mycorrhizal (S or S+B) plants.

Table 1

NH_4-acetate + EDTA-soluble (Lakanen and Erviö, 1971) element content (mg kg^{-1} dry matter) in the calcareous loamy chernozem soil

Element applied	Metal rates originally applied in 1991 (mg metal kg^{-1} dry soil)				$LSD_{5\%}$	Mean
	0	30	90	270		
	mg kg^{-1} dry soil in 1998					
Cd	0.2	13.7	33.0	85.1	7.4	33.0
Ni	2.6	16.3	37.1	51.7	8.0	26.9
Zn	1.3	24.4	60.7	85.7	12.8	43.0

Table 2

Dry matter accumulation of mycorrhizal and non-mycorrhizal ryegrass shoots in 1999 (g pot^{-1})

Element applied	Treatment in 1999	Metal rates originally applied in 1991 (mg metal kg^{-1} dry soil)				$LSD_{5\%}$	Mean
		0	30	90	270		
		mg kg^{-1} dry soil in 1998					
Cd	S	2.26	2.48	1.91	1.82		2.12
	S+B	2.09	2.15	1.98	1.66	0.37	1.97
	S+AMF	2.1	1.79	1.85	1.69		1.86
Ni	S	2.26	2.21	1.94	1.82		2.06
	S+B	2.09	2.0	1.76	1.67	0.35	1.88
	S+AMF	2.1	1.94	1.82	1.79		1.91
Zn	S	2.26	2.0	2.34	2.18		2.20
	S+B	2.09	2.3	2.21	2.02	0.28	2.16
	S+AMF	2.1	2.23	1.92	1.90		2.04

S = sterilised (without AMF or other soil microorganisms); S + B = sterilised + soil suspension (without AMF); S + AMF = sterilised + AMF inoculum (AMF and other soil microorganisms)

The microscopic observation of stained root samples (Phillips and Hayman, 1970) revealed no root colonization by AM fungi either in irradiation-sterilized or in sterilized soil treatments re-inoculated with AMF-free soil suspension. This means that no AMF infection occurred during the experimental period in any of the sterilized treatments. In contrast, high AMF colonization was observed in the sterilized then AMF-inoculated treatments (Table 4). Changes in the parameters of mycorrhizal root colonization were investigated as a function of the metal type and metal rate. The infection frequency of AMF was universally high. The frequency of infection (F%) in ryegrass did not decrease significantly with increasing Cd and Zn rates. The quantity of arbuscules (a%), indicative of the real efficiency of AM symbiosis, decreased at increasing rates of Zn, Cd and Ni application.

Table 3.
Metal content in mycorrhizal or non-mycorrhizal ryegrass roots and shoots in 1999

Element applied	Treatment in 1999	Metal rates originally applied in 1991 (mg metal kg^{-1} dry soil)				$LSD_{5\%}$	Mean
		0	30	90	270		
		mg kg^{-1} dry soil in 1998					
		Metal content, mg kg^{-1} dry roots					
	S	1.2	115	276	629		255
Cd	S+B	1.3	105	193	307	23	151
	S+AMF	0.78	74	158	285		129
	S	15	49	108	156		82
Ni	S+B	13	36	92	155	11	74
	S+AMF	11	31	77	152		68
	S	90	210	233	301		208
Zn	S+B	118	174	322	380	40	248
	S+AMF	53	120	223	287		171
		Metal content, mg kg^{-1} dry shoots					
	S	0.2	5.5	9.8	11.2		6.7
Cd	S+B	0.2	4.3	8.4	10.0	0.96	5.7
	S+AMF	0.1	3.4	7.4	10.9		5.4
	S	5.6	23	29	44.2		25
Ni	S+B	5	16.5	26	32.5	3	20
	S+AMF	2.7	10.7	22	30		16
	S	29	99	195	274		149
Zn	S+B	27	94	159	200	15	120
	S+AMF	16	66	116	160		90

S= sterilised (without AMF or other soil microorganisms); S+B= sterilised + soil suspension (without AMF); S+AMF=sterilised + AMF inoculum (AMF and other soil microrganisms)

Table 4
Extent of AMF infection (F%) and arbuscularity (a%) in ryegrass roots colonized with metal non-adapted AMF in 1999

Element applied	Metal rates originally applied in 1991 (mg metal kg^{-1} dry soil)				$LSD_{5\%}$	Mean
	0	30	90	270		
	Frequency of AMF infection, %					
Cd	56	47	48	38	n.s.	47
Ni	56	50	43	33	13	46
Zn	56	61	59	59	n.s.	59
	Extent of AMF arbuscularity, %					
Cd	10	5.4	1.6	0.5	0.8	4.4
Ni	10	0.5	0.1	1.0	5.8	2.9
Zn	10	0.2	8.3	0.8	6.3	4.8

F% = Frequency of AMF infection in ryegrass roots colonized with metal non-adapted AMF; a% =A rbuscularity of ryegrass roots colonized with metal non-adapted AMF; n.s. = Not significant

Previous studies have reported that AM fungi decrease the metal uptake of the host plants, thus protecting them against heavy metal toxicity (Leyval et al., 1997). Several heavy metal-tolerant AM fungi have been isolated from polluted soils (Gildon and Tinker, 1981; Weissenhorn et al., 1993, 1994). *Glomus mosseae* (BEG69) isolated from a metal-polluted soil was more tolerant to Cd in the spore germination test than *Glomus mosseae* (BEG12) from non-polluted soil (Weissenhorn et al., 1993, 1994). The effect of AMF on the plant uptake of metals is not always clear (Leyval et al., 1997). Reduced metal transport from the soil through the roots to the shoots in the presence of AM fungi has also been reported (El-Kherbawy et al., 1989; Leyval et al., 1997). Joner and Leyval (1997) found that AM mycelium had high cation exchange- and metal sorption capacity and these properties were affected by adaptation to high level of heavy metals.

The present study demonstrated that metal non-adapted AM might colonize perennial ryegrass (*Lolium perenne* L.) and decrease the heavy metal uptake in a soil artificially contaminated by Cd, Ni and Zn. The AMF inoculum used in this study originated from an unpolluted natural ecosystem. The results support the hypothesis that the presence of mycorrhizal symbiosis may decrease metal transport into aboveground plant parts, but this beneficial plant-protecting effect of decreased metal uptake has a price: a significant amount of plant assimilates are transported to the fungi. In mycorrhizal ryegrass (*Lolium perenne* L.) lower biomass production was observed. In summary, the results of this study indicate that plant tolerance to heavy metal stress can be improved by metal non-adapted AMF colonization. The AMF communities of natural ecosystems comprise AMF species, strains or ecotypes which can colonize the host roots, and the presence of symbiosis decreases the harmful metal effects. It can be concluded that the main effect of soil microorganisms, especially AMF, is to increase the stress resistance of higher plants, leading to improved ecosystem stability.

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EVALUATION OF SOIL PEDOTRANSFER FUNCTIONS FOR SOILS OF THE CSALLÓKÖZ AND SZIGETKÖZ REGIONS

V. ŠTEKAUEROVÁ[1], J. ŠÚTOR[1] and C. FARKAS[2]

[1]INSTITUTE OF HYDROLOGY OF THE SLOVAK ACADEMY OF SCIENCES, BRATISLAVA, SLOVAK REPUBLIC
[2]RESEARCH INSTITUTE FOR SOIL SCIENCE AND AGRICULTURAL CHEMISTRY OF THE HUNGARIAN ACADEMY OF SCIENCES, HUNGARY

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In this study pedotransfer functions (PTFs) were developed to estimate the soil water retention curves (SWRCs) for Rye Island (Csallóköz, S. W. Slovakia). A representative set of soil water retention curves was measured using a laboratory method on samples taken from soils the study area. Particle size distribution and bulk density were determined as well. Multiple regression analysis was used for estimating nine statistical relationships in order to predict the drying part of the SWRCs. Texture and bulk density were used as predictors. Pedotransfer functions were verified on another set of measured water retention curves from the same territory as well as on SWRCs determined for soils of the Szigetköz region in Hungary. A good agreement was found between the calculated and measured SWRCs for the Slovakian soils, while somewhat poorer estimates could be given for Hungarian soils.

Key words: pedotransfer function, water retention curve, particle size distribution, bulk density

Introduction

Soil water retention curves (SWRCs) are important hydraulic properties for studying water flow and solute transport in soil. However, their measurement is costly and time-consuming, and requires special equipment. In general, soil particle-size distribution data are more easily measured. Hence, several studies have been carried out during the past 10 years on the statistical estimation of the characteristic points of the SWRC from easily available characteristics (e.g. particle size distribution, bulk density, humus content, organic carbon content, etc.). This method is based on the pre-assumed relationship between the soil water content and soil structure characteristics, as well as on the dependence of bulk density on humus content and organic carbon content. The mathematical expression of this relationship is known as the pedotransfer function (PTF). The importance of PTFs is considerable due to the current application of mathematical models for calculating water movement in the unsaturated zone on a regional scale (Nemes et al., 2003), e.g. with the purpose of:

- Evaluating anthropogenic impacts on soil water balance factors;
- Quantifying ground water pollution;
- Evaluating plant-available water in the unsaturated zone;
- Analysing the influences of slow global changes.

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In practice there is not enough time to measure water retention curves (as one of the basic inputs of mathematical models) by traditional laboratory methods in most cases (Kutílek and Nielsen, 1994). In addition, PTFs enable the use of all pedological information collected previously from a certain area (Pachepsky et al., 1982; Rajkai et al., 1996; Wösten et al., 2001). That is why methods for determining soil hydrophysical characteristics from soil textural and structural parameters were devised in soil physics. Particle size distribution was used at first for this purpose (Brooks and Corey, 1964; Husz, 1967; Renger, 1971), while later bulk density and organic matter content were also utilised as predictors (Gupta and Larson, 1979; Rawls et al., 1982). Studies aimed at determining SWRCs can be grouped according to three tendencies. The authors in the first group (Cosby et al., 1984; Pucket et al., 1985; Vereecken et al., 1989, 1990; Wösten et al., 1995; Wiliams et al., 1983; Bastet et al., 1998) improve methods they have formerly elaborated. The second approach is based on a physical model of the soil-water system, according to the following scheme (Haverkamp and Parlange, 1986; Tietje and Tapkenhinrichs, 1993):

a) Determination of pore-size distribution based on the particle size distribution;

b) Estimation of soil water content from pore size distribution;

c) Calculation of the soil water potential from pore size distribution using mathematical quantifications of capillary phenomena in the soil-water system.

The third approach uses an analytical expression of the water retention curve, such as that of Van Genuchten (1980), i.e. a relationship between soil water content (Θ) and soil water potential (ψ). In this case the individual parameters appearing in the expression are obtained either by regression of their dependency on basic soil characteristics (e.g. particle size distribution, porosity, organic C content, bulk and particle densities and humus content) (Skalová, 2002) or by neural networks (Schaap et al., 2001; Minasny and McBratney, 2002).

At present, the basic aim of PTF determination is to find a PTF expression with general validity for all soils (Tietje and Tapkenhinrichs, 1993; Bastet et al., 1998; Van den Berg et al., 1997; Singh, 1998). General validity is not ascribed to the devised PTF in the present study. The pedotransfer functions created from the soil physical and hydrophysical characteristics of the Rye Island natural environment are applicable with the presented accuracy only for this region.

Materials and methods

Rye Island (called also Žitni Ostrov or Csallóköz) is a narrow island that belongs to the Kisalföld Region and is situated between 47°49' and 48°11' N and 39°49' and 35°49' E, sloping down from NW to SE. The territory of the Kisalföld Region stretches from the Bakony Mountains (Hungary) to the northern side of the Danube and the Tribec Mountains (Slovakia) and includes the geographically and climatically similar Csallóköz and Szigetköz Islands.

The water retention curves of Rye Island soils were used to determine pedotransfer functions for the study area. Between 1990 and 1998 undisturbed soil samples were collected from selected sites on the area, taking into consideration the percentage occurrence of typical soil patterns. The SWRCs were measured in the laboratory with an overpressure apparatus (Soil Moisture Equipment, Santa Barbara, California) at pressure heads representing pF values of 0.3, 1.75, 2.0, 2.3, 2.74, 3.0, 3.48 and 4.2. The soil water content at a pressure head representing pF 4.78 (usually considered as Θ_{res} – residual water content in the analytical expression of Van Genuchten) was determined with an exsiccator method on disturbed soil samples (Šútor and Komár, 1984). In the present paper $\Theta_{res.}$ expresses the water content determined for the hygroscopicity value (Šútor and Majerčák, 1988). The entire dataset included the data of 221 water retention curves. The statistical features of the SWRCs measured for the given pF values are presented in Table 1.

Table 1

Basic statistics of the soil water content set measured at different soil moisture potentials for Slovakian soil samples. Θ_{pF} – water content [100 m^3m^{-3}]

Statistics	$\Theta_{pF=0}$	$\Theta_{pF=1.75}$	$\Theta_{pF=2}$	$\Theta_{pF=2.3}$	$\Theta_{pF=2.74}$	$\Theta_{pF=3}$	$\Theta_{pF=3.48}$	$\Theta_{pF=4.2}$	$\Theta_{pF=4.78}$
Average	42.3	37.0	36.3	34.8	31.4	27.2	25.1	13.4	12.2
Std. deviation	0.36	0.42	0.61	0.45	0.47	0.55	0.38	0.54	0.37
Median	41.31	36.60	37.60	35.00	30.59	27.99	24.66	14.01	11.50
Modus	38.6	35.09	37.00	34.90	29.39	27.43	22.93	4.50	7.50
Variance	28.58	28.58	56.21	40.65	35.77	67.09	23.50	37.24	31.47
Kurtosis	0.30	0.22	1.41	1.19	–0.04	0.20	0.51	–0.65	0.07
Skewness	0.40	0.27	–1.06	–0.44	0.36	–0.37	0.16	–0.09	0.57
Range	35.73	32.95	42.54	42.74	33.29	43.43	28.26	7.05	27.80
Minimum	25.80	21.22	9.66	10.60	16.32	5.79	11.07	0.40	2.00
Maximum	61.53	54.17	52.20	53.34	49.61	49.22	39.33	27.45	29.80
Sample size	221	159	151	199	159	224	159	128	235
Coeff. of variation	0.13	0.14	0.21	0.18	0.19	0.30	0.19	0.46	0.40

Multiple linear regression was applied to find relationships between the soil water content at selected pF values, bulk density ρ_d (g cm^{-3}) and four categories of particle size distribution (I: $d < 0.01$ mm; II: $0.01 \text{ mm} < d < 0.05$ mm; III: $0.05 \text{ mm} < d < 0.1$ mm; IV: $0.1 \text{ mm} < d < 2$ mm, where d is the particle diameter). The main statistical characteristics of the soil texture classes and bulk density used for the multiple regressions are given in Table 2.

For the verification of the PTFs the soil physical data of 24 soil samples from the Rye Island region not included in the multiple regression analyses were used. Thus, the 9 characteristic points of the soil water retention curves were calculated for the 24 reference soil samples (indicated as V1–V24), using the corresponding bulk density data and the particle size distribution categories described above. The verification was based on the comparison of the calculated and measured soil water retention curves. The Van Genuchten analytical expression (Van Genuchten, 1980) was used to represent the shape of the water retention curves for both measured and calculated points.

Table 2
Statistical characteristics of the representative soil physical properties for measured points of water retention curves

N	$\log_{10}\psi$	Stat. char.	Category I [%]	Category II [%]	Category III [%]	Category IV [%]	Bulk density [g cm^{-3}]
1	pF = 0.3	Average	37.36	36.52	17.91	7.96	1.44
		Minimum	16	12	2	0	0.91
		Maximum	66	52	39	42	1.72
		Sample size	221	221	221	221	221
2	pF = 1.76	Average	35.69	38.65	17.62	7.87	1.44
		Minimum	16	22	2	0	0.91
		Maximum	60	52	39	41	1.72
		Sample size	159	159	159	159	159
3	pF = 2	Average	28.00	35.75	27.82	8.27	1.38
		Minimum	4.2	8.51	2.71	0.04	0.97
		Maximum	77.81	76.2	82.32	59.1	1.7
		Sample size	151	151	151	151	151
4	pF = 2.3	Average	37.70	34.63	22.44	4.95	1.44
		Minimum	4.48	8.51	2	0	0.91
		Maximum	77.81	52.78	82.32	41	1.72
		Sample size	199	199	199	199	199
5	pF = 2.74	Average	35.69	38.65	17.62	7.87	1.44
		Minimum	16	22	2	0	0.91
		Maximum	60	52	39	41	1.72
		Sample size	159	159	159	159	159
6	pF = 3	Average	29.10	40.52	20.08	10.26	1.40
		Minimum	4.2	12.3	2	0	0.91
		Maximum	60	76.2	75.13	59.1	1.72
		Sample size	224	224	224	224	224
7	pF = 3.48	Average	35.69	38.65	17.62	7.87	1.44
		Minimum	16	22	2	0	0.91
		Maximum	60	52	39	41	1.72
		Sample size	159	159	159	159	159
8	pF = 4.2	Average	37.99	31.84	25.60	4.13	1.44
		Minimum	4.48	8.51	2.71	0.04	1.00
		Maximum	77.81	52.78	82.32	41	1.70
		Sample size	128	128	128	128	128
9	pF = 4.78	Average	12.18				
		Minimum	2				
		Maximum	29.80				
		Sample size	235				

Evaluation

The mean difference (MD) and the root mean square difference (RMSD) were used to evaluate the accuracy of the estimated SWRCs and the closeness between the measured and calculated water retention curves, respectively, according to Vereecken et al. (1992) and Tietje and Tapkenhinrichs (1993). The MD and RMSD characteristics allow a direct comparison of PTFs, even if they are based on different datasets. Thus, pedotransfer functions, developed by different authors for different sites, can be compared. According to this method, the Van Genuchten analytical expression is fitted to both measured and estimated points of SWRCs within a water potential interval $<a;b> \equiv <-74130$ cm; 0 cm$>$, and the MD and RMSD values are calculated using the following integrals:

$$\mathrm{MD}=\frac{1}{b-a}\int_a^b(\Theta p-\Theta m)d\psi \tag{1}$$

$$\mathrm{RMSD}=\left[\frac{1}{b-a}\int_a^b(\Theta p-\Theta m)^2 d\psi\right]^{1/2} \tag{2}$$

where: $d\psi$ is the water potential increment and Θ_m and Θ_p are the measured and estimated values of the soil water content, respectively. The MD values are either positive or negative, depending on whether the soil water contents calculated from the PTF are higher or lower than the measured ones. They equal zero if the water retention curve of the measured data is identical to that calculated by PTF. On the other hand, MD = 0 does not mean that RMSD = 0 as well. RMSD values determine the closeness between the measured and estimated values of the water retention curves. When reviewing the PTF evaluation of 13 authors, Tietje and Tapkenhinrichs (1993) reported 5 cases studies where the PTFs gave good estimates for 100% of the cases with values: MD=1.29 and RMSD=5.75 (Renger, 1971); MD=0.95 and RMSD=6.11 (Arya and Paris, 1981); MD=–0.19 (Cosby et al., 1988); MD=–5.27 and RMSD=7.51 (Rawls and Brakensiek, 1985); and MD=–1.45 and RMSD=5.31 (Vereecken et al., 1990).

The expansibility of the calculated PTFs for Hungarian soils was tested with the same method, based on the soil physical dataset available for the Szigetköz Region in Hungary. The dataset consisted of particle size distribution and bulk density data from 20 layers of 9 soil profiles as well as of SWRCs (indicated as H1–H20), determined at pF values of 0.0, 0.4, 1.5, 2.0, 2.3, 2.7, 3.4, 4.2 and 6.2. The latter were measured by the method proposed by Várallyay (1973). Table 3 presents the basic statistics for the Hungarian dataset. The residual water content was determined manually from the shape of the water retention curves and water contents corresponding to pF=4.2 and pF=6.2. Values corresponding to 0.01 mm particle diameter were determined manually from the particle size distribution function to avoid disagreement between the diameter partition used in Slovakia (having data at d=0.01 mm) and Hungary (with data at d=0.02 mm). The texture and bulk density data available in the Hungarian dataset were used to calculate the SWRCs from PTFs determined from the Slovakian database, while the measured SWRCs from the Hungarian dataset were used to verify the validity of the PTFs for the Szigetköz Region. The validation was performed by calculating the MD and RMSD values for the analytical (Van Genuchten) functions that were fitted to both the measured and estimated soil water retention curves.

Table 3

Basic statistics of the soil water content set measured at different soil moisture potentials for Hungarian soil samples. Θ_{pF} – water content [100 m^{3}m^{-3}]

Stat. character.	$\Theta_{pF=0}$	$\Theta_{pF=0.4}$	$\Theta_{pF=1.5}$	$\Theta_{pF=2}$	$\Theta_{pF=2.3}$	$\Theta_{pF=2.7}$	$\Theta_{pF=3.4}$	$\Theta_{pF=4.2}$	$\Theta_{pF=6.2}$
Average	48.54	47.08	44.29	42.08	38.17	35.30	31.29	26.06	15.55
Std. deviation	4.53	4.53	4.42	4.30	5.16	5.97	7.15	7.87	6.21
Median	48.31	46.82	44.11	42.60	38.64	35.52	31.17	26.93	15.54
Modus	52.62	49.58	43.69	44.42	43.40	33.93	36.41	27.20	20.77
Variance	20.54	20.48	19.53	18.52	26.62	35.62	51.09	62.01	38.57
Kurtosis	1.14	0.91	2.23	5.21	3.50	1.91	0.21	–0.06	–0.74
Skewness	-0.46	–0.33	–0.74	–1.58	–1.55	–1.21	–0.77	–0.61	0.00
Range	23.85	23.70	25.65	25.65	25.80	27.75	30.20	32.20	24.70
Minimum	58.15	57.10	54.45	49.75	44.70	43.55	42.30	38.80	28.20
Maximum	34.30	33.40	28.80	24.10	18.90	15.80	12.10	6.60	3.50
Sample size	50	50	50	50	50	50	50	50	50
Coeff. of variation	0.09	0.10	0.10	0.10	0.14	0.17	0.23	0.30	0.40

Results and discussion

Equation 3 introduces the general structure of the calculated PTFs. The values of the parameters (a_1, a_2, ... a_6) obtained for different points on the soil water retention curve are given in Table 4 together with the corresponding correlation coefficients (r).

$$\Theta_{pF} = a_1X1 + a_2X2 + a_3X3 + a_4X4 + a_5X5 + a_6 \quad (3)$$

where: Θ_{pF} is the soil water content for pF 0.3, 1.75, 2, 2.3, 2.74, 3, 3.48, 4.2 and 4.78, X1, X2, X3 and X4 stand for the main texture categories I, II, III and IV, respectively, and X5 is the soil bulk density (g cm^{-3}).

The calculated PTFs reflect the physical background of the soil water retention phenomena, which is determined by both structural and textural characteristics. An inverse relationship was found between the bulk density and soil water content at different suctions (Table 4), probably because smaller values of bulk density correspond to greater porosity and relatively higher soil water content. The clay content gave the best prediction of soil water retention in the high suction range ($\Theta_{pF=4.78}$), possibly because the clay content primarily influences the soil microporosity and thus the retention properties of the soil in the high suction range.

It can be concluded that the set of soil properties affecting water retention depends on the range of matric potential. The relative influence of each property varies as well. The relative weight of bulk density, for example, increases as suction decreases (Table 4), probably because soil water retention is influenced by soil structure to a greater extent in the low suction range than in the high suction range.

The lowest correlation coefficient (0.66) was found for the soil pedotransfer function calculated to estimate the soil water content at pF=2, probably because the water retention curves for the studied soils have their inflection point close to field capacity. Moreover, around this suction range the main water driving forces change from gravitational to capillary (Stefanovits, 1992), which makes the estimation of $\Theta_{pF=2}$ more complex. Many authors came to similar conclusions. According to Rawls et al. (1982), sand and clay contents are useful to predict water retention in the low (pF<2) suction range, whereas silt and clay content influence water retention at higher (pF>2) values. It was concluded that $\Theta_{pF=2}$ was a rather difficult property to predict. When attempting to solve this problem, Rajkai et al. (1997) found that field capacity ($\Theta_{pF=2.3}$) was the most effective concomitant variable for predicting the water retention data of Swedish and Hungarian soils and that by including field capacity as a predictor in PDFs the estimation efficiency could be increased significantly. However, data on $\Theta_{pF=2}$ or $\Theta_{pF=2.3}$ are not available in most soil physical databases.

Table 4

Parameter values (a_1, a_2, ... a_6) and correlation coefficients (r) of the pedotransfer functions developed for Rye Island. Predictive properties X1–X4 correspond to main textural categories

	Parameters						
	a_1	a_2	a_3	a_4	a_5	a_6	r
	Corresponding predictive soil property						
	X1	X2	X3	X4	X5		
$\Theta_{pF=0.3}$	0.1940	0.1090	0.0491	0.1225	–29.9873	72.2029	0.80
$\Theta_{pF=1.75}$	0.0739	0.1689	–0.2136	0.0352	–21.3444	62.0236	0.74
$\Theta_{pF=2.0}$	–0.0009	0.0257	–0.1527	–0.3334	–7.2780	52.5034	0.66
$\Theta_{pF=2.3}$	0.0195	0.0019	–0.2066	–0.0470	–21.8808	70.3349	0.72
$\Theta_{pF=2.74}$	0.0530	0.1594	–0.2932	–0.0182	–23.5785	62.5331	0.77
$\Theta_{pF=3.0}$	–0.0650	–0.0905	–0.5955	–0.3002	–12.6870	65.5509	0.72
$\Theta_{pF=3.48}$	–0.0714	–0.1803	–0.4179	–0.26570	–15.5787	66.4839	0.74
$\Theta_{pF=4.2}$	0.0966	0.0488	–0.1420	0.2650	–8.8446	23.3886	0.70
$\Theta_{pF=4.78}$	0.2285	0	0	0	0	–0.2607	0.88

(X1: $d < 0.01$; X2: $0.01 < d < 0.05$; X3: $0.05 < d < 0.1$; X4: $0.1 < d < 2$; where d (mm) is the particle diameter); X5 is the soil bulk density (g cm^{-3}).

Table 5 shows the MD and RMSD values calculated for the Slovakian soils according to equations (1) and (2). Three groups of water retention curves were identified for the Slovakian soils. The groups include the SWRCs of 13, 5 and 6 soil samples, with RMSD values of 0 to 0.3, 0.3 to 0.6 and 0.6 to 1.0 m^3m^{-3}, respectively. The Van Genuchten model fitted to some of the measured and calculated soil water retention curves of the first, second and third groups are shown in Figures 1, 2 and 3, respectively.

The MD and RMSD values determined for Hungarian soils were generally higher than those of the Slovakian ones (Table 6). Figures 4–6 illustrate the measured and estimated SWRCs for the first (RMSD between 0 and 0.4 m^3m^{-3}), second (RMSD: 0.4 to 0.8 m^3m^{-3}) and third (RMSD: 0.8 to 1.2 m^3m^{-3}) groups of curves.

The differences between the measured and estimated SWRCs can be attributed to several causes. It is well known that water retention depends on soil parameters in a complex manner. On the other hand, not all the soil properties influencing water retention in soils were taken into consideration (e.g. humus and organic carbon contents were not included in the calculation), because only the soil physical properties commonly available in soil physical datasets were used. In spite of the fact that only texture and bulk density data were used for Slovakian soils, the PTFs obtained could be used for prediction with relatively high efficiency. This can be explained by the small spatial variability of the soil physical properties not taken into consideration in the Rye Island region. Hence, the influence of these properties on the SWRCs at all sites in the study area is approximately the same.

Table 5
Mean difference (MD in m^3m^{-3}) and root mean squared difference (RMSD in m^3/m^{-3}) values for comparison of measured and estimated values of soil water retention curves on Slovak soils

Site	MD m^3m^{-3}	RMSD m^3m^{-3}	Site	MD m^3m^{-3}	RMSD m^3m^{-3}	Site	MD m^3m^{-3}	RMSD m^3m^{-3}
V1	0.60	0.06	V9	−5.34	0.54	V17	−2.41	0.25
V2	2.12	0.21	V10	1.18	0.12	V18	−0.77	0.09
V3	−6.38	0.66	V11	−7.17	0.72	V19	5.27	0.55
V4	1.69	0.17	V12	−5.14	0.54	V20	−2.10	0.22
V5	2.88	0.29	V13	−3.40	0.39	V21	−2.70	0.30
V6	−8.94	0.90	V14	−1.61	0.16	V22	1.73	0.19
V7	−9.31	0.95	V15	−1.36	0.14	V23	−6.55	0.66
V8	−4.18	0.42	V16	−0.12	0.02	V24	−7.78	0.78

Table 6
Mean difference (MD in m^3m^{-3}) and root mean squared difference (RMSD in m^3m^{-3}) values for comparison of measured and estimated values of soil water retention curves on Hungarian soils

Site	MD m^3m^{-3}	RMSD m^3m^{-3}	Site	MD m^3m^{-3}	RMSD m^3m^{-3}
H1	−6.26	0.63	H11	−11.70	1.17
H2	−2.45	0.26	H12	3.95	0.40
H3	0.85	0.12	H13	−6.95	0.70
H4	−2.67	0.27	H14	−5.26	0.54
H5	−6.15	0.62	H15	−3.58	0.36
H6	−4.53	0.46	H16	−1.81	0.19
H7	−10.80	1.09	H17	−6.16	0.63
H8	−7.32	0.74	H18	−6.16	0.62
H9	−11.00	1.11	H19	−10.10	1.01
H10	−9.11	0.92	H20	−4.23	0.42

In the case of the Hungarian soils, differences were found between the measured and estimated soil water retention curves in the low suction range even for curves where the estimation was the most precise. The water content at saturation was underestimated in all cases. Structural differences between the soils as well as the seasonal variability of soil bulk density and the total volume of macropores might be the explanation for this. On the other hand, pedotransfer functions derived from local datasets give better estimates, in general, than PTFs developed on independent (not local) datasets (Nemes, 2003). Thus, in the same range of matric potentials, the best predictive soil properties vary with the soil type.

When evaluating PTFs from much bigger (European and intercontinental) databases using neural networks, Nemes (2003) obtained RMSD values (from 0.3 up to 0.73), fairly similar to those obtained in the present work and applied the PTFs as input data to simulate the soil water regime of seven Hungarian soils using a dynamic simulation model. The estimated soil water dynamics were compared with those estimated from measured soil water retention curves. The effectiveness of the PTFs was statistically verified. Thus, soil pedotransfer functions evaluated from the Slovakian database can be used to calculate soil physical input data to simulate the soil water regime of the Csallóköz and Szigetköz regions.

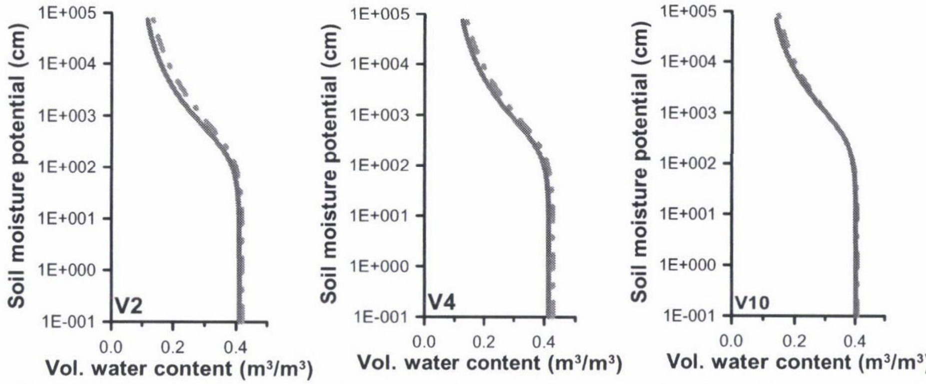

Fig. 1. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Slovakian soils for RMSD (100 m^3m^{-3}) values in the interval 0–0.3

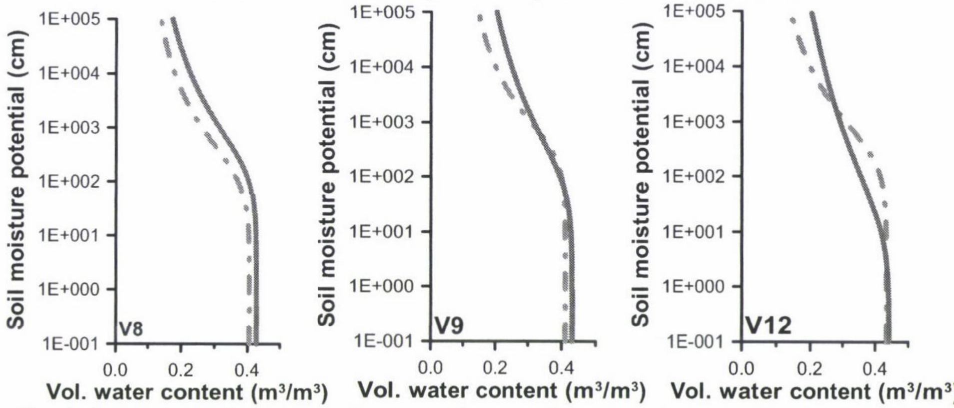

Fig. 2. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Slovakian soils for RMSD (100 m^3m^{-3}) values in the interval 0.3–0.6

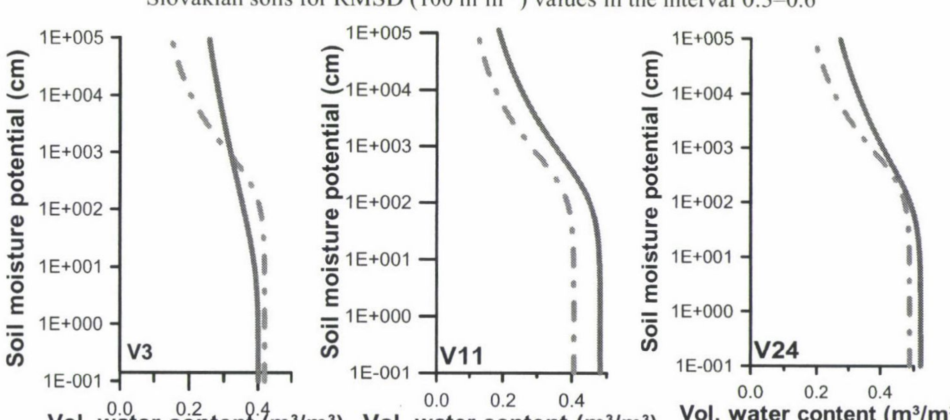

Fig. 3. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Slovakian soils for RMSD (100 m^3m^{-3}) values in the interval 0.6–1.0

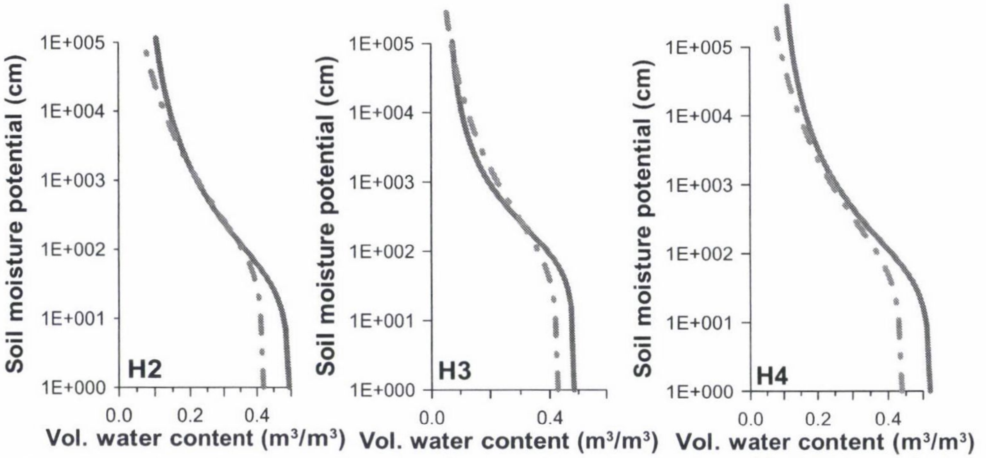

Fig. 4. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Hungarian soils for RMSD (100 m^3m^{-3}) values in the interval 0–0.4

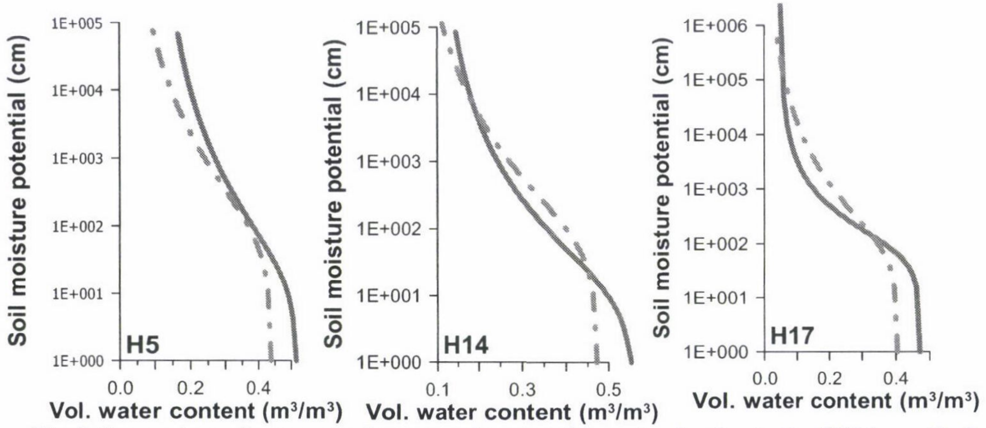

Fig. 5. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Hungarian soils for RMSD (100 m^3m^{-3}) values in the interval 0.4–0.8

Conclusions

Soil hydraulic PTFs were developed to estimate the water retention characteristics of soils in the Rye Island (Csallóköz) natural environment. The PTFs obtained, corresponding to 9 points on the water retention curve, were verified on both local and independent sets of soil physical properties.

The calculated PTFs reflect the physical background of the soil water retention phenomena. The relative influence of each predictive soil property varied, depending on the range of the soil matric potential. The relative weight of bulk density, for example, increased as suction decreased, while clay content was the main property which predicted soil water retention in the high suction range ($\Theta_{pF=4.78}$).

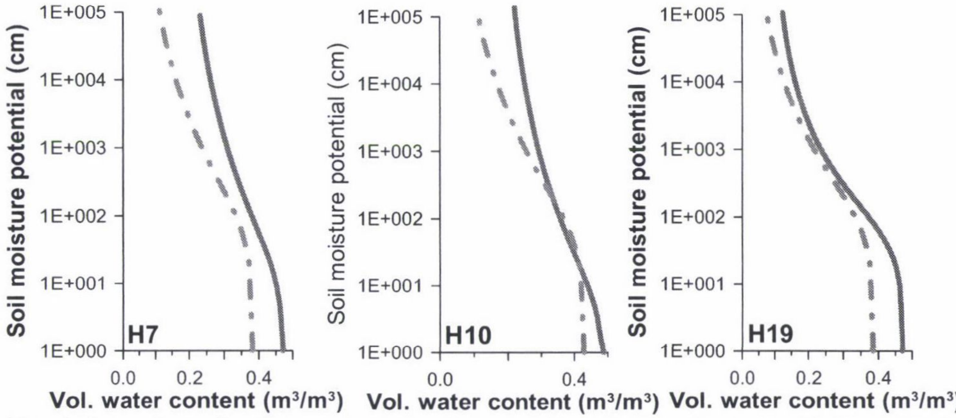

Fig. 6. Comparison of water retention curves [measured (——) and estimated by PTF (– – –)] of Hungarian soils for RMSD (100 m^3m^{-3}) values in the interval 0.8–1.2

A good agreement was found between the calculated and measured SWRCs for Rye Island (Csallóköz), while somewhat poorer estimates could be given for the Szigetköz region. Thus, the use of a set of relevant (local) data is better than using an independent dataset.

It was concluded that the PTFs created could be used as inputs for mathematical models to calculate the soil water regime of the Csallóköz (Rye Island) and Szigetköz regions.

Acknowledgements

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Book review

A. R. OVERMAN and R. V. SCHOLTZ III. *Mathematical Models of Crop Growth and Yield.* Marcel Dekker, 270 Madison Avenue, New York, NY 10016. 2002. Hardcover, 344 pp., $ 150.00. ISBN 0-8247-0825-3.

Mathematical Models of Crop Growth and Yield examines the response of seasonal dry matter to applied nutrients, considers the accumulation of dry matter and plant nutrients, discusses water availability, explores sensitivity analysis, evaluates coupling among applied, soil and plant components, presents numerical procedures for regression analysis and supplies exercises for further study. The book brings together Allan Overman's work on developing equations to describe relationships between crop growth, crop nutrient uptake, amounts of nutrients applied, and time. The approach to modelling in this book is very much motivated and guided by experimental results.

By the nature of the subject, this book necessarily involves many equations of different types. The functions are all of the analytical type, in contrast to numerical techniques. Throughout, the authors follow the practice of starting with simpler models and then progressing towards greater levels of complexity and comprehensiveness. The reader is encouraged to scan the book to view data and model simulations before trying to master mathematical details. Data are presented in both tabular and graphical form in several cases to help the reader obtain a more comprehensive grasp of the material. Throughout the book, each development of the equations is evaluated by showing how the functions fit experimental observations and about one-third of the book consists of exercises inviting the reader to determine model parameters and fit the equations to data.

Chapter 1 (Introduction) deals with the relationship of dry matter yield to amounts of applied nutrients. The authors show that the logistic function can be used to describe the response of crops to applied N, P and K. A justification is provided for the logistic model over the Mitscherlich model for relating dry matter production to applied nutrients. A growth model (probability model) developed by Overman was used to simulate dry matter accumulation and nutrients with time. This chapter is somewhat of an introduction to several models. Succeeding chapters deal with these models at increasingly complex levels. The idea is to develop the motivation for learning the mathematical concepts by demonstrating the practical application of the model.

In *Chapter 2 (Seasonal Response Models),* it is shown how the logistic model can be extended to cover plant nutrient uptake as well as dry matter production, while dry matter yield tends to be a hyperbolic function of N uptake. The next challenge was to expand the mathematical models to incorporate dry matter and plant nutrient accumulation as related to multiple levels of applied nutrients (N, P, K) in a mathematically self-consistent manner. A multiple logistic model was developed for this purpose.

In *Chapter 3 (Growth Response Models)* the empirical growth model is expanded to a phenomenological model with a physical basis, using a growth rate equation (differential equation) as the product of a Gaussian environmental function and a linear intrinsic (genetic) growth function. This model was shown to apply to warm-season perennial grass for harvest intervals up to about 6 weeks, but failed for longer growth intervals and did not apply to annual grasses such as maize. Eventually, the growth model was modified again to cover these cases.

In *Chapter 4 (Mathematical Characteristics of Models)* attention is focused on mathematical details such as the solution of differential equations and the characteristics of some of the functions. Some readers may wish to skip certain details, which are provided to give a sound mathematical basis for the models.

In *Chapter 5 (Pasture Systems)* the authors examine the linkage of animal production with forage production, and in turn with applied N. Two different mathematical models (quadratic and linear exponential) are discussed.

Overall, the book is very readable. Unusually for this kind of text, it is written in a conversational style. The book may be of interest to crop agronomists, research workers, postgraduate students, and final year undergraduate students in the fields of agronomy, crop physiology, applied botany, biometry and statistics. Although the book assumes only moderate mathematical, statistical and computational expertise, the reader should ideally be a person who has been trained in calculus and differential equations.

Z. BERZSENYI

Floral Biology, Pollination and Fertilisation in Temperate Zone Fruit Species and Grape

Pál Kozma, József Nyéki, Miklós Soltész, Zoltán Szabó

This book fills a gap in the scientific literature of horticulture. A detailed survey has been presented on the topic of floral biology related to the temperate-zone fruit species and grape. Prominent representatives of the profession looked over the abundant stock of knowledge of the international documentation and compared it with their own experimentally checked proofs and life experiences. Earlier, in 1996, an English survey edited by J. Nyéki and M. Soltész appeared on general questions of floral biology, i.e. blooming, pollination, fertilisation and fruit set of fruit species. The present volume is considered to be the second volume, the continuation of the former. Floral biology of temperate fruit species and grape are treated even more intrinsically in addition to the aspects of the principles of planning plantations in order to maximize yield and fruit quality by purposeful association of mutually compatible varieties. Theory and practical knowledge are alloyed in order to equalize biology and technological know how, which is difficult and rarely met in the literature. Text, demonstration and rich illustration are carefully balanced aiming at utilisation of the book in teaching, education and postgradual training, as well. A profuse stock of references will help teachers, researchers and students of the horticultural profession at different levels in orientation.

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ORIGINAL PAPERS

MAGYAR
TUDOMÁNYOS AKADÉMIA
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Acta Agronomica Hungarica, 51(4), pp. 371–380 (2003)

PHYSIOLOGICAL STUDIES ON THE INFLUENCE OF CYTOKININ OR GA_3 IN THE ALLEVIATION OF SALT STRESS IN SORGHUM PLANTS

A. M. ISMAIL

BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, QENA, EGYPT

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Salt stress reduced the germination capacity, the root and shoot lengths, the production of fresh and dry matter, and the water content in sorghum (*Sorghum bicolor* L.) seedlings. This reduction was concomitant with a decrease in the contents of soluble proteins, free amino acids and nucleic acids (RNA and DNA), while proline and quaternary ammonium compounds (QACs) increased, especially at low and moderate (50 and 100 mM NaCl) salinity levels.

Pre-soaking sorghum grains in either cytokinin or gibberellic acid (GA_3) partially or completely counteracted the adverse effects of salinity on the rate of germination, seedling growth and some metabolic mechanisms. Generally, exogenous applications of cytokinin or GA_3 enhance the metabolic processes of sorghum plants and improve their tolerance to salt stress.

Key words: *Sorghum bicolor,* cytokinin, gibberellic acid (GA_3), nucleic acids, QACs

Introduction

Salinity is an important ecological factor that creates serious problems for agricultural productivity in many parts of the world (Almansouri et al., 1999). It is common knowledge that salt stress has an adverse effect on the growth rate of plants and consequently on final yield (Ismail, 1996; Glenn et al., 1999). Increasing NaCl levels significantly reduced the percentage germination, dry matter production and soluble proteins, as well as RNA and DNA synthesis (Scutt, 1997; Flowers et al., 2000). This reduction is due to the accumulation of toxic ions and/or reduced water uptake by the root (Munns et al., 1995; Almansouri et al., 2001).

Attempts have been made to employ active phytohormones to overcome the drastic effect of salt stress on plants (Bejaoui, 1985; Singh et al., 1994; Lin and Kao, 1995). The soaking of seeds of responsive cultivars presowing with phytohormones could thus be exploited to ensure better germination and enhanced early seedling growth under salinity stress (Shaddad and Heikal, 1982; Younis et al., 1994).

Therefore, this study attempted to investigate the effect of soaking grains of *Sorghum bicolor* in either cytokinin or gibberellic acid (GA_3) to explain the strategy for alleviating the deleterious effects of NaCl and the enhancement of some metabolic activities.

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Materials and methods

The sorghum (*Sorghum bicolor* L.) grains used in this investigation were obtained from the garden of the Agronomy Department, Faculty of Agriculture, South Valley University in Qena. The salt stresses used were 0.0 (control), 50, 100, 150, 200, 250 and 300 mM NaCl in 1/10 Hoagland solution. Grains of the control group were germinated using only 1/10 Hoagland solution as a substrate. Preliminary screening for various concentrations (50 to 300 ppm) of cytokinin or gibberellic acid (GA_3) was made to obtain the optimum response and a concentration of 100 ppm was selected. To evaluate the interactive effects of phytohormones with salinity, sorghum grains were soaked presowing in either cytokinin or gibberellic acid solution (100 ppm) for 4 hours, then air dried for 48 hours.

Twenty grains of *Sorghum bicolor* were pretreated with 5.25% sodium hypochlorite for 3 minutes. The grains were washed with distilled water three times, and then germinated in Petri dishes (9 cm diameter) at about 28°C. Three replicates were prepared for each treatment. Another experiment was carried out simultaneously to study the interaction between treatments with cytokinin or gibberellic acid and salinity stress on grain germination. The final percentage of germination was recorded after a period of 4 days.

After two weeks of germination the seedling root and shoot lengths were recorded in addition to fresh and dry matter yields. Soluble protein was determined according to Lowry et al. (1951). Free amino acids and proline were determined according to Moore and Stein (1948) and Bates et al. (1973), respectively. Quaternary ammonium compounds (QACs) were determined according to Story and Wyn Jones (1977). RNA and DNA were determined according to Wanka (1962) with some modifications as described by Lukavsky et al. (1973). The fresh matter was extracted 5 times for 50 min at 20°C with 0.2 N perchloric acid in 5% ethyl alcohol and 3 times for 10 min at 70°C with a mixture of ethanol and ether (3:1) then washed with 96% ethyl alcohol. The nucleic acids in the sediment were hydrolysed with 0.5 N perchloric acid at 60°C for 5 h. The total concentration of nucleic acids in the extract was measured by UV absorption at 260 nm. For DNA estimation in the extract, a calorimetric reaction mixture was allowed to stand for 24 h in the dark at 30°C, after which the absorption at 600 nm was measured. The concentration of RNA was evaluated by subtracting the DNA values from the total nucleic acid content.

The data of all the experiments were subjected to analysis by the least significant difference test (L.S.D.).

Results

The data presented in Figure 1 demonstrated that the germination rate of salt-stressed *Sorghum bicolor* grains remained more or less unchanged at the lowest salinization level used (50 mM NaCl). Thereafter, the rate of germination was significantly reduced with a rise in the salinity level, especially at high levels of NaCl, compared with unsalinized seedlings. Treatment with cytokinin or GA_3 (100 ppm) completely alleviated the inhibitory effects of NaCl salinity on the rate of germination of the experimental plants, especially at low and moderate salinization levels. It is noticeable that this stimulatory effect of phytohormones was more pronounced in the case of GA_3 than in the cytokinin treatment.

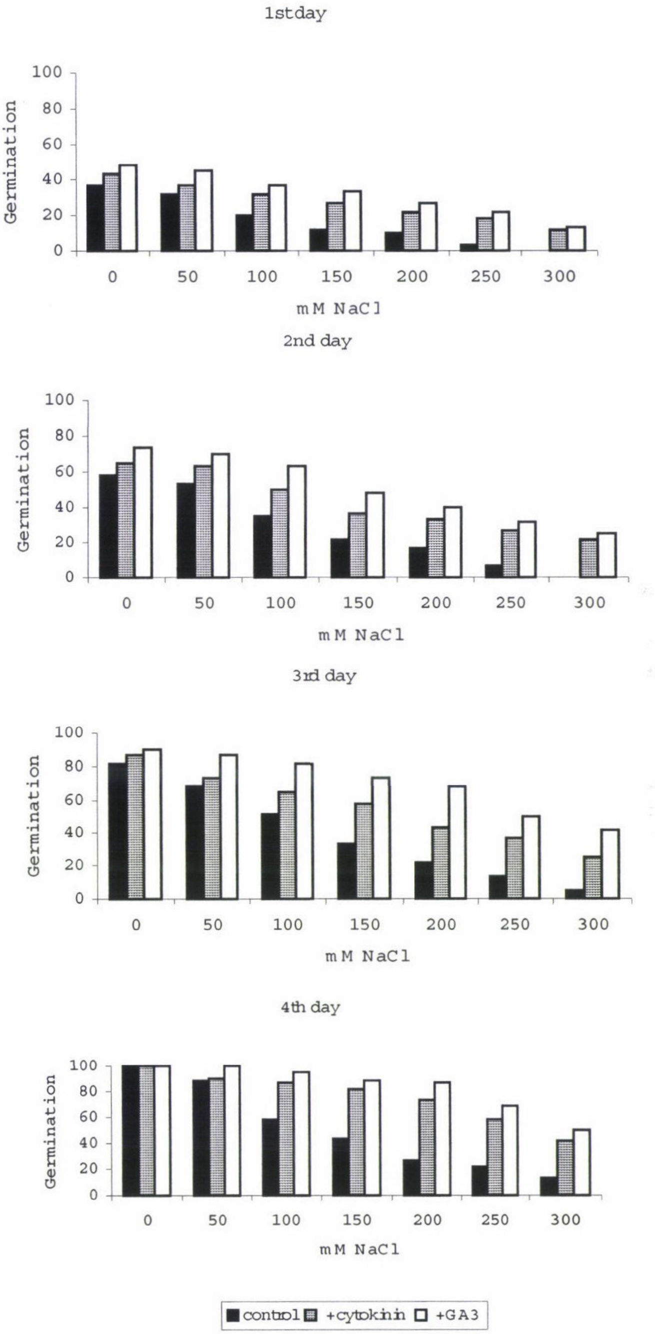

Fig. 1. Effect of cytokinin or gibberellic acid (GA_3) on germination percentage in salinized sorghum grains. Vertical lines indicate least significant differences ($P < 0.01$) for salinity and cytokinin or GA_3 treatments ($n = 3$)

Increasing salinity levels resulted in a significant reduction in the root and shoot lengths of sorghum seedlings (Table 1). This reducing effect was more prominent at high levels of salinity, above 150 mM NaCl. Pre-soaking sorghum grains in cytokinin or GA_3 resulted in a significant increase in each of the root and shoot lengths when compared with the corresponding treatments with only NaCl salinity. Moreover, each of the two phytohormone treatments resulted in greater lengths of roots and shoots than the control plants, especially at low and moderate salinity levels. It is also worth mentioning that the effect of GA_3 on the root and shoot lengths was more obvious than that of cytokinin.

The data presented in Table 2 show that fresh weight and dry matter yield as well as tissue water content were significantly decreased with increasing salt stress compared with unsalinized plants (control). Presoaking of sorghum grains in any of the two phytohormones (cytokinin or GA_3) considerably stimulated the production of fresh and dry matter and the water content in comparison with that of the corresponding seedlings treated with salinity only. This stimulation was more pronounced in GA_3-treated plants than in those treated with cytokinin (Table 2).

Table 1

Effects of cytokinin or gibberellic acid (GA_3) on root and shoot lengths (cm seedling^{-1}) of salinized sorghum seedlings

Treatments	Salinity only		+100 mg/l cytokinin		+100 mg/l GA_3	
NaCl [mM]	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length
0.0	8.90	6.70	12.50	7.60	14.10	8.25
	±0.46	±0.41	±0.35	±0.74	±0.46	±0.58
50	6.45**	5.31*	12.75	7.85	14.85*	9.10*
	±0.93	±0.62	±0.61	±0.67	±0.48	±0.91
100	6.50**	4.92**	11.85	7.75	14.90*	8.35
	±0.27	±0.64	±0.85	±0.34	±0.45	±0.48
150	5.80**	3.30**	9.40**	6.40**	13.75	7.90
	±0.53	±0.30	±0.75	±0.42	±0.30	±0.36
200	2.71**	1.85**	7.55**	5.85**	13.45*	7.45*
	±0.20	±0.23	±0.42	±0.39	±0.47	±0.53
250	2.20**	1.45**	6.50**	3.90**	8.90**	5.85**
	±0.34	±0.18	±0.55	±0.31	±0.35	±0.56
300	1.60**	1.25**	5.70**	3.55**	8.35**	4.60**
	±0.11	±0.16	±0.33	±0.25	±0.61	±0.34
$LSD_{5\%}$	1.47	1.12	0.70	0.63	0.61	0.69
$LSD_{1\%}$	2.01	1.53	0.96	0.86	0.84	0.92

Data are means ± SD of 3 replicates. * = Significant (P = 0.05) and ** = Highly significant (P = 0.01) differences as compared with the control.

Table 2

Effects of cytokinin or gibberellic acid (GA_3) on fresh weight (FW) and dry matter (DM) (g plant^{-1}) and water content (WC, %) of salinized sorghum plants

Treatments	Salinity only			+ 100 mg/l cytokinin			+100 mg/l GA_3		
NaCl [mM]	FW	DM	WC	FW	DM	WC	FW	DM	WC
0.0	5.331	0.601	88.7	7.881	0.703	91.0	8.725	0.718	91.7
	±0.43	±0.04	±0.60	±0.42	±0.014	±0.22	±0.38	±0.024	±0.71
50	4.880*	0.568*	88.3	7.420	0.698	90.5	8.673	0.711	91.8
	±0.50	±0.03	±0.41	±0.48	±0.021	±1.04	±0.14	±0.019	±1.25
100	4.120**	0.558**	86.4**	6.072*	0.577**	90.4	8.061	0.691	91.4
	±0.33	±0.01	±0.70	±0.09	±0.027	±0.51	±0.12	±0.015	±1.20
150	3.743**	0.544**	85.4**	5.313**	0.561**	89.4*	7.003*	0.653	90.7
	±0.18	±0.02	±0.80	±0.11	±0.013	±1.10	±0.42	±0.011	±0.63
200	2.650**	0.425**	83.9**	3.774**	0.506**	86.5**	6.551**	0.627*	90.4
	±0.22	±0.02	±0.70	±0.11	±0.014	±0.62	±0.13	±0.014	±0.60
250	2.160**	0.379**	82.4**	3.022**	0.471**	84.4**	5.076**	0.578**	88.6**
	±0.08	±0.01	±0.70	±0.13	±0.017	±0.70	±0.18	±0.016	±1.37
300	1.570**	0.297**	81.0**	2.792**	0.439**	84.2**	3.785**	0.511**	86.5**
	±0.07	±0.01	±0.45	±0.15	±0.014	±0.91	±0.07	±0.020	±1.24
$LSD_{5\%}$	0.35	0.027	1.10	1.331	0.084	1.18	1.54	0.088	1.60
$LSD_{1\%}$	0.48	0.037	1.51	1.823	0.115	1.62	2.11	0.120	2.19

Data are means ± SD of 3 replicates. * = Significant (P = 0.05) and ** = Highly significant (P = 0.01) differences as compared with the control.

Salinity induced a marked, progressive decrease in the content of soluble proteins in sorghum seedlings, especially at higher salinity levels (Table 3). On the other hand, phytohormone treatments resulted in a significant increase in soluble proteins in salt-treated sorghum seedlings. This increase was more obvious in the case of GA_3 than for cytokinin. The proline content of sorghum seedlings showed progressively greater accumulation, above that of the control, with increasing salt concentration in the culture medium. The opposite pattern was exhibited in the contents of other amino acids, which significantly decreased with a rise in the salinity level, the reduction becoming more obvious at higher salinity levels (Table 3). On the other hand, presoaking sorghum grains in cytokinin or GA_3 also resulted in a stimulation of the accumulation of free amino acids compared with the corresponding untreated plants. By contrast, proline accumulation was obviously inhibited by the phytohormone treatments compared with the corresponding treatments with NaCl only.

Quaternary ammonium compounds (QACs) increased up to the level of 200 mM NaCl, but above this level the values declined compared with those in unstressed plants (Table 3). Phytohormone treatments markedly decreased the accumulation of QACs compared with the corresponding treatments with NaCl only. This effect was more obvious in the case of GA_3 than for cytokinin.

Table 3

Effects of cytokinin or gibberellic acid (GA_3) on the production of soluble proteins, free amino acids, proline and quaternary ammonium compounds (QACs) [mg g^{-1} (d.m.)] in salinized sorghum seedlings

Treatments NaCl [mM]	Soluble protein	Free amino acids	Proline	QACs
	Salinity only			
0.0	10.70 ± 0.82	5.82 ± 0.61	0.753 ± 0.042	4.59 ± 0.43
50	9.65* ± 0.46	4.51** ± 0.37	0.944** ± 0.045	4.88** ± 0.44
100	7.55** ± 0.22	4.08** ± 0.12	1.134** ± 0.013	6.19** ± 0.23
150	5.13** ± 0.22	3.55** ± 0.57	1.664** ± 0.037	6.47** ± 0.37
200	4.35** ± 0.36	3.07** ± 0.39	1.875** ± 0.045	6.27** ± 0.34
250	3.47** ± 0.66	3.11** ± 0.43	1.893** ± 0.018	4.54 ± 0.52
300	3.70** ± 0.31	2.65** ± 0.52	1.955** ± 0.075	4.47** ± 0.35
$LSD_{5\%}$	0.83	0.56	0.122	0.20
$LSD_{1\%}$	1.14	0.77	0.167	0.27
	+100 mg/l cytokinin			
0.0	13.45 ± 0.68	6.15 ± 0.22	0.611 ± 0.025	2.88 ± 0.30
50	14.72* ± 0.82	6.71 ± 0.18	0.632 ± 0.035	2.93 ± 0.25
100	16.20** ± 0.39	6.24* ± 0.31	0.607 ± 0.027	2.67** ± 0.14
150	17.75** ± 0.85	8.33** ± 0.23	0.565* ± 0.025	2.04** ± 0.18
200	19.33** ± 0.43	10.12** ± 1.05	0.513** ± 0.021	1.75** ± 0.13
250	19.71** ± 0.40	10.48** ± 0.73	0.477** ± 0.016	1.41** ± 0.20
300	20.34** ± 0.59	11.25** ± 0.84	0.416** ± 0.047	1.27** ± 0.10
$LSD_{5\%}$	1.09	1.04	0.038	0.11
$LSD_{1\%}$	1.50	1.42	0.052	0.15
	+100 mg/l GA_3			
0.0	17.80 ± 0.69	7.32 ± 0.33	0.503 ± 0.013	2.91 ± 0.17
50	19.20* ± 0.94	7.85 ± 0.64	0.464* ± 0.086	2.17** ± 0.28
100	22.41** ± 0.29	8.35* ± 0.55	0.443** ± 0.042	1.88** ± 0.33
150	24.70** ± 0.42	10.14** ± 0.87	0.379** ± 0.080	1.67** ± 0.26
200	27.11** ± 0.31	11.54** ± 0.98	0.361** ± 0.012	1.46** ± 0.31
250	27.84** ± 0.83	12.27** ± 0.37	0.288** ± 0.022	1.37** ± 0.28
300	28.21** ± 0.79	12.36** ± 0.78	0.274** ± 0.014	1.04** ± 0.16
$LSD_{5\%}$	1.24	0.89	0.034	0.09
$LSD_{1\%}$	1.70	1.22	0.046	0.12

Data are means ± SD of 3 replicates. * = Significant (P = 0.05) and ** = Highly significant (P = 0.01) differences as compared with the control.

The data in Table 4 reveal that the DNA content of salt-treated sorghum seedlings was significantly lower than that of the control plants, irrespective of the level of salt. The content of RNA remained more or less unaffected up to the level of 100 mM NaCl, but above this level the values decreased more sharply than found in the control seedlings. Treatment with phytohormones (cytokinin or GA_3) resulted in a marked, progressive increase in the contents of both DNA and RNA in sorghum seedlings compared with the corresponding untreated ones. This increase was more pronounced in the case of GA_3 than for cytokinin, especially at higher levels (250 and 300 mM) of NaCl.

Table 4

Effects of cytokinin or gibberellic acid (GA_3) on the contents of DNA and RNA [$\mu g/g^{-1}$ (f.w.)] in salinized sorghum seedlings

Treatments	Salinity only		+100 mg/l cytokinin		+100 mg/l GA_3	
NaCl [mM]	RNA	DNA	RNA	DNA	RNA	DNA
0.0	14.10 ±0.35	11.75 ±0.29	20.26 ±0.85	13.35 ±0.29	26.35 ±0.95	19.55 ±0.71
50	14.30 ±0.65	9.05** ±0.56	20.47 ±0.88	13.75 ±0.35	26.80 ±0.89	19.21 ±0.33
100	12.86 ±0.78	8.77** ±0.82	19.84 ±0.58	11.30** ±0.34	26.61 ±0.97	18.55 ±0.46
150	9.80** ±0.49	8.20** ±0.16	17.93** ±0.75	10.77** ±0.31	26.15 ±0.44	17.85* ±0.41
200	9.70** ±0.90	7.75** ±0.42	16.15** ±0.93	9.25** ±0.90	25.62* ±0.56	12.77** ±0.27
250	6.33** ±0.68	3.91** ±0.12	13.65** ±0.61	7.83** ±0.25	21.37** ±0.71	11.79** ±0.80
300	6.50** ±0.39	3.43** ±0.24	11.80** ±0.65	6.40** ±0.46	21.41** ±0.93	10.66** ±0.75
$LSD_{5\%}$	1.27	0.81	1.21	1.03	0.79	1.28
$LSD_{1\%}$	1.74	1.11	1.66	1.41	1.08	1.75

Data are means ± SD of 3 replicates. * = Significant (P = 0.05) and ** = Highly significant (P = 0.01) differences as compared with the control.

Discussion

It appears from the present study that salinity resulted in a significant reduction in seed germination, root and shoot lengths, and fresh and dry matter production in *Sorghum bicolor* seedlings. This reduction was found to be concomitant with lower values of tissue water content. This inhibitory effect of salinity may be attributed to the accumulation of toxic ions and reduced water uptake which arrests radicle emergence (Dodd and Donovan, 1999; Almansouri et al., 2001). In this respect, Chazen and Neumann (1994) reported that the reduction in dry matter yield may be explained by cell wall hardening and limited extension growth.

Pre-soaking sorghum grains in either cytokinin or GA_3 alleviated the suppressive effects of salinity on the seed germination, root and shoot lengths, and dry matter yield of the experimental plants. It is interesting to note here that the highly promoting effect of the two phytohormones appeared at relatively higher salinity levels. This alleviation was more pronounced in the case of GA_3 than for cytokinin. In connection with this, Dhingra and Varghese (1985) and Lin and Kao (1995) reported that GA_3 plays a primary role in the induction of germination and in the release of dormancy. Moreover, Jupe et al. (1988) and Rood et al. (1990) suggested that GA_3 can stimulate growth by increasing cell elongation in some plant species, and by increasing cell elongation and cell division in others. On the other hand, Katsumi (1962) reported that cytokinin stimulated growth by increasing the diameter rather than the elongation, while Fox (1964) found that cytokinin usually inhibited the elongation of stem sections. In this respect, the cytokinin-induced release of lateral buds from inhibition by the apical bud coincided with the growth and juncture of xylem elements differentiating basipetally from the bud within the stem internode. This establishment allowed further growth and development because of the increased water and solute supply (Wilkins, 1984).

The accumulation of organic solutes under saline conditions has been well documented. In the present work, salinity induced a progressive decrease in the amount of soluble proteins in the tested seedlings. However, at higher salinity levels the losses in soluble proteins in sorghum seedlings were accompanied by an increase in proline and quaternary ammonium compounds (QACs). This indicated that salinity might stimulate the conversion of both soluble proteins and amino acids into proline (Dubey, 1994; Schubert et al., 1995; Ismail and Azooz, 2002). Also, salt reportedly promotes the accumulation of proline together with quaternary ammonium compounds (QACs), which serve as compatible cytoplasmic solutes to maintain the osmotic balance under conditions of water stress (Ramanjulu and Sudhakar, 1997). This supports the theory that the accumulation of organic solutes may reflect the damage caused by NaCl.

Thus, the marked increases in soluble proteins after soaking sorghum grains with either cytokinin or GA_3 may indicate that phytohormones are able to alleviate the imposed salt stress via the stimulation of protein synthesis. In accordance with this, gibberellins and cytokinins play an important role in the treated plants and appear either to form a sink mobilizing the various nutrients which are involved in building new tissues (Luckwill, 1977) and/or to enhance the photosynthetic mechanism and the protein synthesis (Wilkins, 1984). After the application of phytohormones, the accumulation of proline and quaternary ammonium compounds was considerably retarded whatever the phytohormone used. This reduction was concomitant with a promotion in the growth of the test plants. It can thus be concluded that both the phytohormones used are able to alleviate the adverse effects of salt stress. This conclusion is in accordance with the results obtained by Imamul-Huq and Larher (1983).

The present results showed that salinity induced a significant decrease in the content of nucleic acids (DNA and RNA) of sorghum seedlings as the salinity level was raised. In this respect, the inhibition of protein synthesis under salt stress conditions is caused by an increase in the RNase activity (Arad and Richmond, 1976), which evidently affects the rate of protein synthesis by destroying the mRNA linking the ribosomes. On the other hand, the marked increases in the DNA and RNA contents of the phytohormone-treated plants may also indicate that phytohormones are able to alleviate the depressive effects of salt stress. This response was more pronounced in the case of GA_3 than for cytokinin.

Generally, it can be said that both the phytohormones are capable of enhancing plant growth under osmotically stressful conditions. This enhancement could be achieved by improving the water balance via a lowering of the proline content. Both the protein content and the level of nucleic acids were also raised, and this was reflected in the growth and dry matter production of the test plants. In other words, the depressive effects of salt stress on seed germination and seedling growth and on other relevant physiological activities can be alleviated and/or modified to some extent by pre-soaking the seeds in the appropriate concentrations of cytokinin or gibberellic acid.

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NITRATE REDUCTASE ACTIVITY AND YIELD OF *LENS CULINARIS* SPRAYED WITH 28-HOMOBRASSINOLIDE

S. HAYAT and A. AHMAD

PLANT PHYSIOLOGY SECTION, DEPARTMENT OF BOTANY, ALIGARH MUSLIM UNIVERSITY, ALIGARH, INDIA

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Thirty-day-old plants of *Lens culinaris* (L.) Medic. cv. Pusa-6 were sprayed with 10^{-10}, 10^{-8} or 10^{-6} M aqueous solutions of 28-homobrassinolide (HBR). Root length and nodule number per plant decreased, whereas the leaf nitrate reductase activity (E.C. 1.6.6.1) at 60, 90 and 120 days after sowing and the seed yield at harvest increased significantly in plants sprayed with either concentration of HBR. The values increased at first with an increase in the concentration of HBR but decreased with a further increase above 10^{-8}M, which proved best for improving seed production.

Key words: brassinosteroids, nitrate reductase activity, root length, root nodule, protein, seed yield

Introduction

Brassinosteroids (BRs) are natural plant growth regulating substances with wide distribution in the plant kingdom. However, their exogenous application to plants has a growth promotive effect (Mandava, 1988; Sakurai and Fujioka, 1993). Similarly, they have been demonstrated to have a wide spectrum of physiological roles in plants, that includes stem elongation, pollen tube growth, leaf bending and epinasty, root growth inhibition, the induction of ethylene biosynthesis, proton pump activation, xylem differentiation, the regulation of gene expression (Mandava, 1988; Clouse and Sasse, 1998; Li and Chory, 1999; Khripach et al., 2000) and an increase in carbonic anhydrase and nitrate reductase activities (Hayat et al., 2001a, b). Moreover, BRs have also been employed for economic gains, since treated plants develop stress resistance and produce more seeds in the majority of crops (Cutler et al., 1991; Hayat et al., 2000, 2001b; Hayat and Ahmad, 2003). In contrast to the above, rooting and subsequent growth have been reported to give a negative response to 24-epibrassinolide (Roddick and Guan, 1991) and brassinolide (Adam and Marquardt, 1986; Jones-Held et al., 1996) in various plants or their organs. The present work included observations on the nodule-bearing capacity and some related aspects of nitrogen metabolism in plants of *Lens culinaris* sprayed with 28-homobrassinolide, which was selected because it is more stable under field conditions (Khripach et al., 2000).

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Materials and methods

The seeds of *Lens culinaris* (L.) Medic cv. Pusa-6 were purchased from the National Seed Corporation Ltd., New Delhi, India.

This experiment was conducted during the winter seasons of 2000–2001 and 2001–2002 at the agricultural farm of the Aligarh Muslim University, Aligarh. The soil was sandy loam (pH 7.8). Surface sterilized (0.1% mercuric chloride solution) healthy seeds were sown in 5 m^2 plots at the rate of 50 kg/ha. These seeds were uniformly inoculated with *Rhizobium* sp. The plants were sprayed with water (control), or 10^{-10}, 10^{-8} or 10^{-6} M aqueous solutions of 28-homobrassinolide (HBR) at day 30. Each treatment comprised five replicates. Root length and nodule number were studied at 60, 90 and 120 days after sowing (DAS). The leaf nitrate reductase activity was estimated in fresh samples at 60, 90 and 120 DAS following the method of Jaworski (1971). This method is based on the reduction of nitrate to nitrite, which was determined colorimetrically. Various yield attributes, including pod number plant^{-1}, length pod^{-1}, seed number pod^{-1}, 1000 seed weight and seed yield were studied at harvest (i.e. 140 DAS).

The seed proteins in the homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 1% sodium hydroxide solution. The method of Lowry et al. (1951) was employed to quantify the proteins. The data of both the years were pooled and statistically analysed using analysis of variance at the 5% probability level (Gomez and Gomez, 1984).

Results and discussion

The leaves of plants sprayed with 28-homobrassinolide (HBR) possessed a higher level of nitrate reductase (NR) activity than the water-sprayed control (Fig. 1). However, the level of the enzyme decreased with the age of the plants from 60–120 days after sowing. The maximum increase in NR activity in 60, 90 and 120 day old plants sprayed with 10^{-8} M HBR were 21, 38 and 44 %, respectively over the control. The total nitrate reducing capacity of the plants, according to Campbell (1999), is not only dependent on (a) the availability of the substrate at the level of the cytoplasm, (b) the level of functional NR and (c) the activity level of functional NR, but also on its relation with the overall state of the plant metabolism, where coordination is operated through the sensors and/or signal transducters. Other hormones, including HBR in wheat (Hayat et al., 2001a) and *Lens culinaris* (Hayat and Ahmad, 2003), GA_3 and/or cytokinin in tobacco (Roth-Bejerano and Lips, 1970), IAA in pea (Ahmad, 1988; 1994) and chloroindole auxins in pea cuttings (Ahmad and Hayat, 1999) and mustard seedlings (Ahmad et al., 2001) are also reported to enhance the level of NR. Moreover, an increased level of nitrate, the inducer of the enzyme (Hewitt and Afridi, 1959) could be an additional reason for an increase in NR activity. These nitrate ions essentially induce functional NR by producing "nitrate sensing" proteins of unknown nature which presumably bind with the regulatory regions in the NR genes and turn on the expression of NR-mRNA (Redinbaugh and Campbell, 1991) and the synthesis of other DNA-regulating proteins involved in the metabolic response, leading to morphological changes, for instance in the root/shoot ratio (Redinbaugh and Campbell, 1991; Crawford, 1995; Scheible et al., 1997).

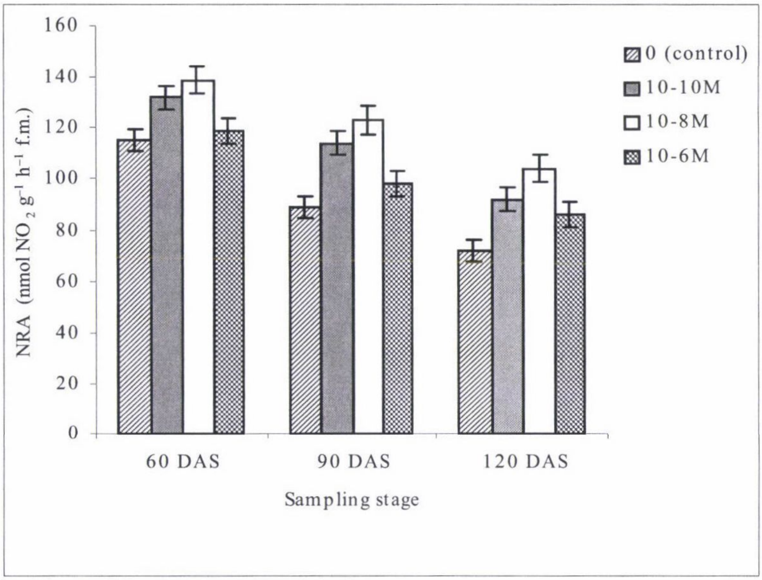

Fig. 1. Nitrate reductase activity at three stages of growth in plants of *Lens culinaris* sprayed with three concentrations of 28-homobrassinolide

The roots of plants, sprayed with HBR were shorter and possessed fewer nodules per plant than the control (Figs. 2 and 3). The degree of inhibition increased with an increase in the concentration of HBR. This alteration in the growth pattern of the root and in nodulation may simply be an expression of the changed pattern of biochemical reactions either with the direct involvement of the genes or via an extra-genetic route. That is most likely to be the regulation of the hormone level, in particular that of auxin, by acting at the level of its synthesis and/or transport. These indirectly affect nodule formation (Kefford et al., 1960; Hunter, 1987; 1989; Hirsch et al., 1989; Fukuhara et al., 1994; Wu et al., 1996) and the partitioning of photosynthates (Brenner and Cheikh, 1995). This is further supported by the synergistic response observed to HBR and auxin (Mandava, 1988). The shoot, therefore, retains a larger share of photosynthates at the expense of the root, and these are made available to the inflorescence axis, resulting in the observed increase in pod number, 1000-seed weight and seed yield at harvest (Table 1). The changes in pod length, seed number and seed protein content were statistically non-significant (Table 1). The 10^{-8} M concentration of HBR proved best, where the above characteristics increased by 22, 4 and 16% over the control, respectively. Similarly, BR (Mandava, 1988; Meudt, 1993) and HBR (Bhatia and Kaur, 1997; Hayat et al., 2000; 2001b) increased the yield characteristics and seed yield of various other crops, which is probably an expression of improved fruit set and grain filling, as also proposed earlier (Luo et al., 1986) in wheat.

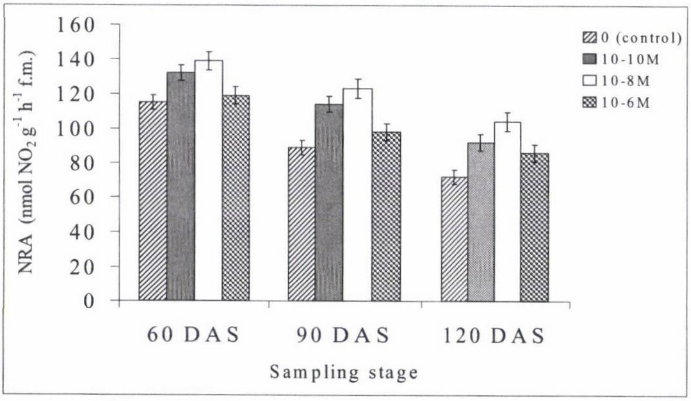

Fig. 2. Root length at three stages of growth in plants of *Lens culinaris* sprayed with three concentrations of 28-homobrassinolide

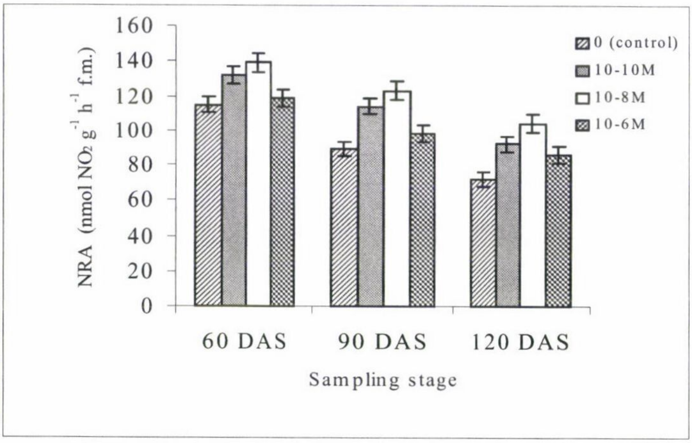

Fig. 3. Root nodule number at three stages of growth in plants of *Lens culinaris* sprayed with three concentrations of 28-homobrassinolide

Table 1

Yield characteristics, seed yield and seed protein content at harvest in plants of *Lens culinaris* sprayed with 28-homobrassinolide (HBR) at day 30

HBR (M)	Pod number plant^{-1}	Pod length (cm)	Seed number pod^{-1}	1000-seed weight (g)	Seed yield (q ha^{-1})	Seed protein content (%)
0 (control)	92.4	0.86	1.80	20.70	13.72	20.42
10^{-10}	112.5	0.91	1.83	21.25	16.05	20.65
10^{-8}	118.4	0.91	1.82	21.65	16.46	20.59
10^{-6}	96.2	0.89	1.81	20.95	15.10	20.48
$LSD_{5\%}$	5.5	N.S.	N.S.	0.24	0.36	N.S.

N.S. = Non-significant

It may be concluded from these observations that HBR primarily had an impact on the general metabolism of the plant and on the partitioning of the photosynthates. A larger share of the photosynthates was probably directed towards the inflorescence axis, at the expense of the roots. This resulted in an improvement in the yield components and the seed yield, but the root growth and nodulation were adversely affected.

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WINTER HARDINESS OF DURUM WHEAT IN HUNGARY

P. SZŰCS, O. VEISZ, G. VIDA and Z. BEDŐ

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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One basic precondition for the reliable cultivation of winter durum wheat (*Triticum durum* Desf.) in Hungary is for the varieties to have good winter hardiness and frost resistance. Field overwintering experiments carried out in Martonvásár between 1995 and 2003 demonstrated that there was a significant difference every year between the overwintering of varieties with poor and good frost resistance, though only in two years was there a significant difference between that of varieties with medium and better frost resistance. Only a medium correlation was observed between the mean annual values of the air temperature in the winter months and the winter hardiness of the varieties, confirming that winter hardiness is influenced jointly by a number of environmental factors (e.g. cold, snow cover). In the experiments carried out on the winter hardiness dynamics of durum wheat, it was found that in milder winters even *T. durum* varieties which are sensitive to frost overwintered with little damage, while in the two coldest winters during the experimental period the hardiness of these varieties did not provide sufficient protection even in December, and all the plants were destroyed by January. The early spring frosts experienced in 1996 proved in these experiments that spring frosts may cause considerable damage even to durum wheat varieties with relatively good winter hardiness. Averaged over eight years, the results prove that *T. durum* genotypes are now available whose average state of hardening and winter hardiness are equal or better than those of winter *T. aestivum* varieties with moderate frost resistance.

Key words: hardening, *Triticum turgidum* L. ssp. *durum*

Introduction

One of the most decisive factors in the reliable production of winter cereals, including durum wheat, is resistance to extreme climatic conditions in winter. In the Carpathian Basin the winter weather conditions may fluctuate considerably, and winters with far milder or more severe weather than average may equally occur. Hard winters may result in substantial yield losses, so breeders are faced with the task of developing winter-hardy winter cereal varieties capable of surviving the most severe winters occurring in the given region with a low level of damage.

Field tests have shown that winter hardiness is a dynamic plant trait which fluctuates considerably during the process of overwintering as a function of environmental factors. Temperatures near the freezing point lead to an increase in the level of hardening during the autumn, and maximum winter hardiness may be achieved by wheat plants in December, January or February (Veisz and Rajki, 1984). One of the most important factors in the overwintering of cereals is the development stage of the plants in early winter. The sowing date must be chosen in such a way that the winter cereals reach at least the three-leaf stage by the

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time winter sets in. The simplest tests of winter hardiness are field experiments where the field-grown plants are exposed to natural growth, hardening and freezing conditions. However, counting the number of frost-killed plants per unit area in the field in spring only provides a good estimation of winter hardiness if the winter was cold and snow-free or if there were extreme temperature fluctuations in early spring. One method often used for the estimation of winter hardiness is to sow the wheat varieties in wooden boxes placed on the soil surface or sunk into the soil in the field. The determination of frost damage takes place in the course of or at the end of the winter. The winter weather needs to be severe on average if testing is to be successful, as in mild winters field observations do not allow frost resistance to be determined or the role of individual factors to be directly investigated (Veisz and Rajki, 1987).

Hungary can be regarded as the northern limit of *T. durum* wheat production in Europe. The frost resistance and winter hardiness of durum wheat has a great influence on whether the species can be grown reliably on large areas in Hungary. Breeders have systematically developed new winter durum varieties surpassing the old spring types in both yield and yield stability (Beke and Matuz, 1996; Szunics et al., 1998). The winter hardiness of the first Hungarian winter durum varieties was poorer than that of the bread wheat varieties cultivated in Hungary, so further improvements were required in their frost resistance and winter hardiness (Beke and Barabás, 1981; Szunics et al., 1987; Szűcs et al., 1998; 1999).

In a series of experiment set up in 1995 the overwintering of a number of winter *T. durum* genotypes was examined over a period of eight years and the dynamics of winter hardiness was investigated under field conditions. The results of these experiments will be presented in this paper.

Materials and methods

The experiments were carried out in the field in Martonvásár between 1995 and 2003. Each year the overwintering of 45 genotypes with breeding value was investigated. In addition to advanced lines, durum wheat, bread wheat and barley varieties of various origins and with different levels of winter hardiness were included as controls. The Odmadur 1, Odmadur 2 and Parus varieties originated from Odessa, Ukraine, the Martondur 1, Martondur 2 and MvTD 32-95 from Martonvásár, GK Tiszadur, GK Bétadur and GK Minaret from Szeged, Hungary, and Zenit and Ares from Bologna, Italy. Two *T. aestivum* varieties with different levels of frost resistance, Bánkúti 1201 (medium) and NS Rana 2 (poor), and the barley variety Kompolti Korai were also sown as controls. The germinated seed were sown in wooden boxes in mid-October each year, with 9 rows of 20 plants in each box, in a random design with four replications. The boxes were placed on the soil surface in the nursery. At the end of the winter the boxes were transferred to the greenhouse and the plants were cut back to a height of 1.5 cm. After two weeks of further growth, plants which had overwintered and exhibited new growth could be clearly distinguished from those which had been killed. To evaluate winter hardiness, the number of overwintered plants was expressed as a percentage of the plant number prior to the winter. In addition to determining the winter hardiness values, the winter hardiness dynamics of nine genotypes was investigated each year. This experiment differed from that described above in that the number of plants surviving in the field was determined each year in early December, January, February, March and April. The results were statistically analysed using two-factor analysis of variance.

Results and discussion

Winter temperature data between 1995 and 2003

Over the last eight years winter bread wheat (*T. aestivum* L.) has not suffered any significant frost damage on a country-wide scale. The monthly air temperature means recorded during the field experiments (Table 1) reveal substantial differences in the mean temperatures of the winter months in the different years. The winter temperatures were mild in 1997/98 and 2000/01, when there were no long periods with temperatures below freezing point. The lowest mean temperatures were recorded in the February of 1995/96 and 2002/03 and in the December of 1998/99 and 2001/02, but even in these cases the mean monthly temperature of the soil in boxes placed on the soil surface did not drop below –4.5°C. Averaged over the years, the mean temperature of the individual months was highest in March and lowest in January. The February mean temperature exhibited the greatest fluctuation over the years, and the January value the least. In the field experiments the soil temperature in boxes placed on the soil surface followed the air temperature and was only a few degrees warmer than the air temperature even during the coldest periods. Due to the snow cover the soil temperature was not very low even in the coldest months, so even winter cereals with greater frost sensitivity were able to overwinter during the last eight years, with only a low extent of frost kill in two winters.

Over the last eight years, the cold winters of 1995/96 and 2002/03 deserve special mention from the point of view of cereal overwintering. Due to repeated falls of snow from November 1995 onwards, the wheatfields were still covered with snow during the first ten days of March 1996. According to data provided by the National Meteorological Service, there were 63 days with snow cover during this winter, which is almost twice the many years' average. The lowest temperature was around –10°C, but a snow cover of up to 30–40 cm in depth protected the crops from any great extent of freezing out. However, the ice layer formed due to freezing rain and the detracted thaw led to more serious losses due to snow mould (*Microdochium nivale*) than had been experienced at any time over the last two decades.

In February 2003 it was extremely cold almost throughout the month, with a mean temperature in Martonvásár of –4.5°C, five degrees lower than the many years' average. The National Meteorological Service data indicated that the daily mean temperatures in February were lower than the many years' average every day. During the winter of 2002/03 extreme cold was experienced not only in February, but also in December and January. The first snow came early, at the beginning of November, but this rapidly thawed, and December was very cold. Thick snow (40–60 cm) covered the fields by the middle of January, and more heavy snow fell in early February. The National Meteorological Service data show that heavy snow was experienced more frequently than average in Hungary between 1994 and 2003.

Table 1
Mean monthly air temperatures (°C) during the winter (Martonvásár, 1995/96–2002/03)

Month	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	Mean	Deviation
November	–0.9	6.6	4.8	2.9	2.9	8.3	3.6	7.1	4.4	2.75
December	5.1	–1.8	2.0	–3.5	0.3	2.1	–3.7	–1.4	–0.1	2.85
January	–3.1	–2.6	2.1	–0.5	–1.3	0.8	1.2	–2.5	–0.7	1.84
February	–3.6	1.3	5.1	0.6	3.9	3.6	5.4	–4.5	1.5	3.55
March	1.1	4.1	4.2	6.9	6.4	8.1	8.0	4.2	5.4	2.24
Mean	–0.2	1.5	3.6	1.3	2.3	4.6	2.8	0.6	2.1	1.49

Overwintering of durum wheat varieties between 1995 and 2003

In each year of the field overwintering experiments 45 genotypes were tested, but the majority of these were different every winter. A few durum wheat cultivars and the control bread wheat and barley varieties were tested every year, so these will be used to demonstrate the results of the overwintering trials. The survival percentages were determined at the end of winter, in early April. The durum wheat varieties presented here all have relatively good frost resistance, they have all been, or are still being, commercially grown in Hungary. According to the analysis of variance the two factors (variety, year) and their interaction were significant. Due to the mild winters, no significant difference was observed between the overwintering values of the varieties in many years (Table 2). A comparison of the overwintering figures, averaged over 8 years, indicates that the two bread wheat control varieties did not differ significantly from each other, while the overwintering levels of several durum varieties (Martondur 1, Parus, GK Tiszadur) equalled or exceeded those of the controls. The average overwintering percentages of the varieties were lowest in 1995/96 and 2002/03. The heavy snow layer which covered the boxes for a long period in the cold winter of 1995/96 led to the appearance of snow mould, as in the field-grown crops, with the result that plant mortality was more than 50% higher by the end of winter than in the following years. The only exception was the barley variety Kompolti Korai, which exhibited a higher overwintering percentage in this year than in any other year of the experiment.

In the cold winter of 2002/03 the survival percentage averaged over the ten varieties did not different significantly from the average recorded in 1995/96, but there were substantial differences between the survival percentages of different varieties in the two cold winters. The varieties Odmadur 1, Odmadur 2, Martondur 1 and Parus survived the winter significantly better in 2002/03 than in 1995/96. The thick, long-lasting snow cover in the winter of 2002/03 provided adequate protection for varieties with better frost resistance, and no snow mould damage was observed. The complete stand of Kompolti Korai barley and half the two bread wheat stands were destroyed during the course of the winter, indicating that there were considerable differences between the tested varieties in the genetically determined frost resistance and snow mould resistance.

Table 2
Winter hardiness of cereals (overwintering %) in boxes placed on the soil surface (Martonvásár, 1995/96–2002/03)

Variety	1995/96	1996/97	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03	Mean	Deviation
Odmadur 1	11.4	64.3	81.4	92.0	73.6	86.6	98.6	79.1	73.4	25.4
Odmadur 2	13.0	72.0	78.3	95.0	74.7	86.4	97.4	61.4	72.2	25.1
Martondur 1	28.0	83.3	97.5	96.0	88.4	91.8	91.0	68.3	80.5	21.6
Martondur 2	5.3	54.5	36.3	91.1	78.3	91.6	86.0	2.7	55.7	34.8
Parus	17.6	93.7	94.9	90.7	86.4	89.1	89.6	52.0	76.8	25.8
GK Tiszadur	23.7	84.2	92.4	91.4	85.7	87.8	91.2	14.0	71.3	30.5
GK Bétadur	18.8	50.2	88.7	95.0	61.4	92.7	92.7	9.2	63.6	32.5
Bánkúti1201	65.1	87.2	87.6	100.0	75.5	80.5	94.9	54.7	80.7	14.2
NS Rana 2	73.8	84.1	81.2	93.6	67.7	90.0	90.9	59.4	80.1	11.4
Kompolti K.*	92.5	73.3	88.8	89.7	85.0	86.4	74.2	0.0	73.7	28.6
Mean	34.9	74.7	82.7	93.4	77.6	88.3	90.7	40.1	72.8	21.3

*Kompolti K.: Kompolti Korai; $LSD_{5\%}$=19.5 between any two values, 6.9 between variety means and 6.2 between year means

A moderately close correlation was observed between the air temperature during the winter months and the average winter hardiness of the varieties each year (r=0.74*). When the relationship between winter hardiness and the mean temperatures in individual months was examined, the closest correlation (r=0.86**) was found in February and the weakest (r=0.27) in November. The air temperature in December was in medium negative correlation (r=–0.49) with the survival of the varieties, indicating that a mild December tended to cause a deterioration in winter hardiness. Among the varieties the winter hardiness of the two Szeged varieties exhibited the closest correlation (GK Tiszadur r=0.91** and GK Bétadur r=0.85**) with the February air temperatures. The fact that only moderate correlations were generally found between winter hardiness and air temperatures proves that winter hardiness is influenced jointly by a number of environmental factors (e.g. cold, snow cover).

Dynamics of winter hardiness of durum genotypes between 1995 and 2003

The characterisation of frost resistance necessitates a knowledge of how this trait changes over time. In experiments on the dynamics of winter hardiness seven winter durum wheat varieties were tested, which differed from each other for this trait and were representative of the whole variety collection. The frost resistance of the tested varieties was evaluated once a month throughout the winter. From the winter hardiness data of the two control *T. aestivum* varieties it was clear that, with the exception of 1995/96 and 2002/03, the environmental factors during the experimental winters were not critical for wheat overwintering. In milder winters even *T. durum* varieties which were more sensitive to frost overwintered without any great loss. One example of this was the winter of 1998/99, when no significant mortality was observed for the majority of tested genotypes (Table 3). Only the winter hardiness of the Italian variety Zenit, which had poor frost resistance, declined substantially from

January onwards, but even so over 80% of the plants of this variety survived the winter. In this year the winter hardiness of the other Italian variety, Ares, was only significantly lower than that of the other varieties in December and April.

The colder winter in 1995/96 and the infection with snow mould had a substantial effect on the overwintering of *T. durum* (Table 4). The winter hardiness of the majority of genotypes was greatest in December, when the varieties achieved the highest level of hardening. After the mild December the hardiness of the plants decreased, leading to a higher ratio of killed plants in the January evaluation, a trend which continued in February and March for most of the genotypes. The hardiness of the two Italian wheat varieties, which had poor winter hardiness, declined substantially by January and the stand was completely destroyed by the end of the winter. The mean temperature was below freezing point from the last ten days of February to mid-March, while the snow mould damage further reduced the survival percentages of the varieties in the evaluation made in early April.

The long, cold winter of 2002/03 caused a sharp reduction in the survival percentages of the control bread wheat varieties as well as those of durum wheat (Table 5). The average winter hardiness of the genotypes in the individual months was greatest in early December, exhibiting a considerable decrease in January. Thanks to the long-lasting snow cover, the winter hardiness of the varieties did not change significantly during the rest of the winter, even after the extremely cold February. The survival percentages of the weakly frost-resistant Italian varieties (Ares, Zenit) were very low even in December, and the two stands were completely destroyed by January. The monthly mean survival percentages were lower in 2002/03 than in 1995/96, except for the April figures. When the survival percentages were averaged for each variety, there was not such a clear relationship between the figures of the two years. A comparison of the winter hardiness data is complicated by the fact that snow mould infection was observed in 1995/96 but not in 2002/03.

Table 3

Dynamics of winter hardiness (overwintering %) of wheat varieties in boxes placed on the soil surface (Martonvásár, 1998/99)

Genotype	December	January	February	March	April	Mean
Odmadur 1	100.0	100.0	100.0	100.0	100.0	100.0
Martondur 1	97.2	94.4	97.1	97.1	87.8	94.7
MvTD 32-95	100.0	94.4	100.0	88.5	94.7	95.5
GK Tiszadur	100.0	93.8	100.0	100.0	91.2	97.0
GK Minaret	100.0	94.7	100.0	97.4	91.9	96.8
Zenit	96.9	83.2	82.4	83.8	80.1	85.3
Ares	82.2	97.1	94.4	97.1	78.5	89.9
Bánkúti 1201	100.0	100.0	100.0	100.0	94.7	98.9
NS Rana 2	100.0	95.0	100.0	95.0	100.0	98.0
Mean	97.4	94.7	97.1	95.4	91.0	95.1

$LSD_{5\%}$=13.7 between any two values, 6.1 between variety means, 4.5 between year means

Table 4
Dynamics of winter hardiness (overwintering %) of wheat varieties in boxes placed on the soil surface (Martonvásár, 1995/96)

Genotype	December	January	February	March	April	Mean
Odmadur 1	97.2	82.0	62.8	60.1	14.7	63.4
Martondur 1	96.4	97.4	91.2	87.5	32.4	81.0
MvTD 32-95	100.0	97.4	90.8	88.6	23.7	80.1
GK Tiszadur	97.2	89.5	78.1	53.9	42.8	72.3
GK Minaret	94.4	63.2	57.0	45.6	29.4	57.9
Zenit	50.6	13.8	10.0	0.0	0.0	14.9
Ares	54.6	0.0	0.0	0.0	0.0	11.0
Bánkúti 1201	93.8	87.5	74.4	71.7	45.1	74.5
NS Rana 2	87.5	81.7	87.5	100.0	71.2	85.6
Mean	85.8	68.0	61.3	56.4	28.8	60.1

$LSD_{5\%}$=19.8 between any two values, 10.3 between variety means, 6.9 between year means

Table 5
Dynamics of winter hardiness (overwintering %) of wheat varieties in boxes placed on the soil surface (Martonvásár, 2002/03)

Genotype	December	January	February	March	April	Mean
Odmadur 1	100.0	73.5	88.9	81.0	82.3	85.1
Martondur 1	91.7	70.0	84.6	66.5	59.4	74.4
MvTD 32-95	78.6	31.8	82.4	80.0	70.0	68.6
GK Tiszadur	61.2	7.6	50.0	50.0	52.8	44.3
GK Minaret	80.0	2.8	5.6	10.5	17.8	23.3
Zenit	57.5	0.0	0.0	0.0	0.0	11.5
Ares	61.7	0.0	0.0	0.0	0.0	12.3
Bánkúti 1201	74.7	46.9	61.1	74.4	75.0	66.4
NS Rana 2	67.2	19.5	51.1	65.2	54.4	51.5
Mean	74.7	28.0	47.1	47.5	45.7	48.6

$LSD_{5\%}$=20.5 between any two values, 13.6 between variety means, 10.2 between year means

Among the winters included in the present experiment, significant differences in the winter hardiness of Hungarian winter *T. durum* genotypes could be detected under the meteorological conditions in 1995/96 and 2002/03. The effect of the early spring frosts in 1996 proved the truth of the practical observation that it is spring rather than winter frosts that cause the greatest damage to durum wheats. The results confirmed that the evaluation of frost resistance for the selection of *T. durum* wheat varieties and other genotypes with good frost resistance and winter hardiness should include tests carried out under controlled conditions in the phytotron (Szunics et al., 1987; Szűcs et al., 1998; 1999). Averaged over eight years, the results prove that *T. durum* genotypes are now available whose average state of hardening and winter hardiness are equal or better than those of winter *T. aestivum* varieties with moderate frost resistance.

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SUBSTITUTION ANALYSIS OF SEEDLING STAGE COPPER TOLERANCE IN WHEAT

A. F. BÁLINT[1], G. KOVÁCS[1], A. BÖRNER[2], G. GALIBA[1] and J. SUTKA[1]

[1]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY
[2]INSTITUT FÜR PFLANZENGENETIK UND KULTURPFLANZENFORSCHUNG, GATERSLEBEN, GERMANY

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The relatively copper-tolerant wheat variety Chinese Spring (recipient), the copper-sensitive variety Cappelle Desprez (donor) and their substitution lines were screened for copper tolerance in a soil pot experiment under artificial growth conditions. Chromosomes 5A, 5B, 5D and 7D of Cappelle Desprez significantly decreased the copper tolerance of the recipient variety to varying extents. By contrast, the 6B and 3D chromosomes significantly increased the copper tolerance of Chinese Spring, suggesting that a wide range of allelic differences could be expected between wheat genotypes for this character. The significant role of homologous group 5 in copper tolerance was confirmed by testing wheat-rye substitution lines. The substitution of rye chromosome 5R (5R/5A substitution line) into a wheat genetic background significantly increased the copper tolerance of the recipient wheat genotype. The results suggest that chromosomes 5R and 5A probably carry major genes or gene complexes responsible for copper tolerance, and that the copper tolerance of wheat can be improved through the substitution of a single chromosome carrying the responsible genes. At the same time, it is also possible that the effect of homologous group 5 is not specific to copper tolerance, but that the genes located on these chromosomes belong to a general stress adaptation (frost, cold, vernalisation requirements, etc.) complex, which has already been detected on this chromosome. To answer this question further studies are needed to determine the real effect of these chromosome regions and loci on copper tolerance.

Key words: copper tolerance, wheat, substitution analysis, chromosomal location

Introduction

Copper (Cu) is one of the most important micronutrients, but at high concentration it is toxic to plants. Due to intensive human activities (mining, agricultur, etc.) Cu pollution has become an increasing problem worldwide (Delas, 1963; Csathó, 1994). However, plants growing in natural Cu-enriched soils may also show serious toxicity symptoms (Lanaras et al., 1993). The selection of more tolerant genotypes could provide gene sources for the breeding of copper-tolerant wheat, while varieties which accumulate high concentrations of copper in their shoots could be directly used for the phytoremediation of polluted soils (Raskin et al., 1997). Despite the fact that the morphological and physiological aspects of copper toxicity in wheat are an intensively investigated area (Eleftheriou and Karataglis, 1989; Lanaras et al., 1993; Ciscato et al., 1997; Landjeva et al., 1998; Quartacci et al., 2000; 2001; Tari et al., 2002), the genetics of copper tolerance in wheat is not well understood. Copper tolerance was associated with chromosomes 5A, 4D and 7D in a study on Chinese

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Spring/*Triticum spelta*, and with chromosomes 5A, 7A and 7B using Chinese Spring/'Synthetic' (*T. diccoccum*/*Ae. squarrosa* amphiploid) substitution lines (Manyowa, 1989). Unfortunately, no genetic data are available on copper tolerance in bread wheat. The identification of chromosome(s) influencing Cu tolerance in bread wheat is complicated by the fact that no really copper-tolerant wheat genotype has yet been discovered, despite the screening of several wheats and wheat relatives. When seedlings of 27 *Aegilops*, *Triticum*, *Secale* and triticale genotypes were screened for copper tolerance in hydroponics, the *Secale* genotypes showed the highest level of tolerance. The majority of the common wheat varieties were sensitive to copper stress, but slightly tolerant genotypes were also identified (Bálint et al., 2002). These two groups of genotypes gave significantly different physiological responses under copper stress conditions (Tari et al., 2002). The results suggested that there was a sufficiently high level of genetic polymorphism for copper tolerance to allow the relevant chromosome(s) to be identified using substitution analysis. To find the most suitable genetic material, the parents of 4 different wheat substitution series (Chinese Spring/Hope, Chinese Spring/Cheyenne, Chinese Spring/Cappelle Desprez, Chinese Spring/*T. aestivum* ssp. *spelta*) were screened for copper tolerance in hydroponics and pot experiments. According to the results the varieties Chinese Spring and Cappelle Desprez showed the greatest significant difference (Bálint et al., 2003).

Based on previous results the aim of the present studies was to determine the chromosome(s) that influence copper tolerance by analysing Chinese Spring/Cappelle Desprez substitution lines. As the *Secale* genotypes were the most copper tolerant in previous experiments (Bálint et al., 2002), the effect of certain rye chromosomes in a wheat genetic background was also tested.

Materials and methods

Plant material

Seeds of the substitution series *Triticum aestivum* ssp. *aestivum* cv. Chinese Spring/Cappelle Desprez were obtained from the Cereal Genebank, Martonvásár, Hungary. Seeds of the wheat/rye substitutions were obtained from the Resources Genetic and Reproduction Group, Gatersleben, Germany. The lines used in the experiment are listed in Tables 1 and 3.

Screening for copper tolerance

The plants were tested in soil pot experiments in growth chambers, using control and copper-treated soil. The copper concentration was 1500 mg/kg $CuSO_4 \times 5\ H_2O$ for the Chinese Spring/Cappelle Desprez substitution and 1000 mg/kg for the wheat-rye substitution lines, as suggested by Bálint et al. (2003). Two weeks after germination the shoot lengths and shoot dry weights were determined. The ratios of shoot lengths and shoot dry weights under stress and non-stress conditions (relative shoot length and relative shoot dry weight) were used as a Tolerance Index (Bálint et al., 2002).

Statistical analysis

The whole experiment was replicated 3 times in independent growth chambers and 5 plants from each replication were analysed. The significance of differences between mean values was determined by one-way analysis of variance. Correlations between the investigated parameters were established by calculating simple pair-wise correlation coefficients.

Results

Shoot lengths under non-stress and stress conditions

In the case of shoot length no differences could be observed between the recipient and donor wheat varieties under non-stress conditions, while the differences were significant under stress conditions. The recipient Chinese Spring showed a relatively high level of copper tolerance, while the chromosome donor Cappelle Desprez was very sensitive to the copper concentration applied. This suggests that Chinese Spring/Cappelle Desprez substitution lines could be useful in identifying the chromosomal location of genes responsible for copper tolerance. Comparing the effect of the individual chromosome substitution lines, only chromosome 5B showed significantly shorter shoot length under stress conditions (Table 1). Interestingly, the 6A chromosome considerably increased the shoot length of the recipient variety under non-stress conditions. Based on the original data set, no other chromosomes with a significant effect were detected. However, if the Tolerance Index (TI) was calculated from the shoot length data, several other chromosomes showed a significant effect. The two varieties differed significantly from each other in respect of TI. The chromosomes of homologous group 5 (5A, 5B and 5D) and 7D significantly decreased the tolerance of the recipient parent. Interestingly, another chromosome was found, 6B, which had a highly significant reverse effect, considerably increasing the copper tolerance of the variety Chinese Spring.

Shoot dry weights under non-stress and stress conditions

No differences were observed between the donor and recipient varieties for the shoot dry weight of the control and copper-treated variants, although the dry weight of the sensitive donor variety was considerable lower under stress conditions. Under non-stress conditions chromosomes 6A and 1B significantly increased the dry weight of the recipient genotype, but no special chromosome effects could be detected under stress conditions in the case of dry weight accumulation (Table 1).

Relative shoot dry weights (Tolerance Index calculated from dry weight data)

Chromosomes 5A, 6B, 3D, 5D and 7D were found to have a significant effect on tolerance, and there was also a significant difference between the parents in respect of tolerance (Table 1).

Correlation between the parameters investigated

There was a significant correlation between the mean values of shoot lengths and shoot dry weights under stress and non-stress conditions (Table 2). The mean values of Tolerance Indexes calculated from shoot lengths also correlated well with those calculated from shoot dry weights. The mean values of the Tolerance Indexes were correlated with the shoot length and shoot dry weight data recorded under stress conditions.

Table 1
Shoot lengths and shoot dry weights of wheat genotypes 14 days after germination under non-stress and stress conditions and the Tolerance Indexes calculated from relative shoot length and relative shoot dry weight data

Genotypes	1	2	3		4	5	6	
			Means	%			Means	%
CS	37.0	30.9	0.84	100.0	0.0423	0.0337	0.80	100.0
CD	35.5ns	26.2ns	0.74**	88.1	0.0443ns	0.0269	0.61**	76.3
CS/CD 1A	36.5ns	31.0ns	0.85ns	101.2	0.0467ns	0.0345	0.74ns	92.5
CS/CD 3A	36.3ns	32.2ns	0.89ns	106.0	0.0441ns	0.0349	0.79ns	98.8
CS/CD 4A	35.2ns	30.4ns	0.86ns	102.4	0.0453ns	0.0359	0.79ns	98.8
CS/CD 5A	37.3ns	27.7ns	0.74**	88.1	0.0470ns	0.0279	0.59*	73.8
CS/CD 6A	39.9*	31.7ns	0.79ns	94.0	0.0488*	0.0384	0.79ns	98.8
CS/CD 7A	37.4ns	31.3ns	0.84ns	100.0	0.0453ns	0.0347	0.77ns	96.3
CS/CD 1B	38.2ns	33.7ns	0.88ns	104.8	0.0478*	0.0399	0.83ns	103.8
CS/CD 3B	38.2ns	32.4ns	0.85ns	101.2	0.0431ns	0.0321	0.74ns	92.5
CS/CD 4B	37.6ns	31.9ns	0.85ns	101.2	0.0426ns	0.0322	0.76ns	95.0
CS/CD 5B	34.7ns	27.1*	0.78*	92.9	0.0407ns	0.0287	0.71ns	88.8
CS/CD 6B	34.5ns	31.4ns	0.91**	108.3	0.0399ns	0.0369	0.92*	115.0
CS/CD 7B	36.0ns	30.2ns	0.84ns	100.0	0.0444ns	0.0341	0.77ns	96.3
CS/CD 1D	37.3ns	30.1ns	0.81ns	96.4	0.0472ns	0.0333	0.71ns	88.8
CS/CD 2D	37.5ns	33.2ns	0.89ns	106.0	0.0441ns	0.0362	0.82ns	102.5
CS/CD 3D	38.1ns	33.5ns	0.88ns	104.8	0.0437ns	0.0432	0.99**	123.8
CS/CD 5D	37.4ns	28.4ns	0.76*	90.5	0.0455ns	0.0282	0.62**	77.5
CS/CD 6D	38.5ns	32.7ns	0.85ns	101.2	0.0455ns	0.0384	0.85ns	106.3
CS/CD 7D	37.4ns	28.2ns	0.75**	89.3	0.0439ns	0.0254	0.58***	72.5

1: Means of control shoot length (cm); 2: Means of treated shoot length (cm); 3: Tolerance Index (Calculated from shoot length data); 4: Means of control shoot weight (g); 5: Means of treated shoot weight (g); 6: Tolerance Index (Calculated from dry weight data); Means of four independent experiments. Means denoted by *, ** and *** are significantly different from cv. Chinese Spring at the $P \leq 0.05$, 0.01 and 0.001 levels, respectively; ns: not significant. Abbreviations: CS: cv Chinese Spring; CD: cv. Cappelle Desprez.

Table 2
Correlation coefficients between copper tolerance traits measured on the Chinese Spring/Cappelle Desprez substitution series

Investigated parameters	Correlation coefficient
Control shoot lengths–control dry weights	0.6206 **
Treated shoot lengths–treated dry weights	0.8705 ***
TI based on shoot lengths–TI based on shoot dry weights	0.8824 ***
Control shoot lengths–TI based on shoot lengths	–0.0091 ns
Control dry weights–TI based on shoot dry weights	–0.1739 ns
Treated shoot lengths–TI based on shoot lengths	0.8508 ***
Treated dry weights–TI based on shoot dry weights	0.9131 ***

** and ***: significant at the $P \leq 0.01$ and 0.001 levels, respectively; ns: not significant. Abbreviation: TI: Tolerance Index.

Effect of rye chromosomes 1R and 5R on copper tolerance in a wheat background

Rye chromosome 5R (5R/5A substitution line) caused an increase in copper tolerance, while rye chromosome 1R (1R/1A substitution line) had no effect on tolerance (Table 3).

Table 3
Tolerance Index of copper-treated wheat and wheat/rye substitution lines 14 days after germination based on the shoot dry weight data

Genotypes	Means of Tolerance Index (Calculated from shoot dry weight data)	SE	Means as a % of Sarat29 means [=means$_{app.}$/(means$_{Sarat29}$/100)]
cv. Saratovskaya 29	0.71	± 0.02	100.0
Sarat29/Viet 5R(5A)	0.91 **	± 0.13	128.2
Sarat29/Viet 1R(1A)	0.71 ns	± 0.04	100.0

Means and ± SE of three independent experiments. Means denoted by ** are significantly different from *T. aestivum* ssp. *aestivum* cv. Saratovskaya 29 at the $P \leq 0.01$ level; ns: not significant. Abbreviations: Sarat29: cv Saratovskaya 29, Viet: *Secale cereale* cv. Vietnamskaya.

Discussion

Identification of chromosomes for copper tolerance

The Chinese Spring/Cappelle Desprez substitution series was selected for the substitution analysis of copper tolerance in wheat, because the parents showed a significant difference in respect of copper tolerance, Chinese Spring being relatively tolerant and Cappelle Desprez relatively sensitive. Under non-stress conditions chromosome 6A was found to have a significant effect on the shoot length and shoot dry mass production (Table 4). Chromosome 1B was also found to affect shoot dry mass production. Under copper stress the shoot length was influenced by chromosome 5B. Tolerance Indexes based on the relative shoot lengths and relative shoot dry weights were used to determine the copper tolerance of the substitution series. The results of the study suggest that the relative shoot dry weight is the most suitable parameter for the determination of tolerance, since greater, more significant effects were found in most cases than when using the relative shoot length data (Table 4). However, there was a significant correlation between the shoot length and shoot dry weight data (Table 2). Chromosomes 5A, 5B, 6B, 3D, 5D and 7D were found to have a significant effect on copper tolerance (results summarized in Table 4). Chromosomes 5A, 5B, 5D and 7D from the relatively sensitive Cappelle Desprez caused a decrease in tolerance in the relatively tolerant Chinese Spring, while chromosomes 6B and 3D caused a significant increase. In homologous group 5 the greatest effect on shoot dry weight and shoot length was detected for chromosomes 5A and 5D. Interestingly, chromosome 5B had only a slight effect on tolerance, although this chromosome was the only one which had an influence on the shoot length under stress conditions. Manyowa (1989) also reported the effect of chromosome 5A on copper tolerance using common wheat/*T. spelta* and common wheat/Synthetic (*T. dicoccum*/*Ae. squarrosa* amphiploid), and found an effect exerted by chromosome 7D when testing wheat/*T. spelta* substitution lines. The role of homologous group 5 is supported by the observation that in rye, genes for increased Cu tolerance are located predominantly on chromosome 2R, while genes with smaller effects are located on chromosome 5R (Manyowa, 1989). These results were confirmed in the present work, because rye chromosome 1R in a wheat background was ineffective, while rye chromosome 5R caused increased tolerance in wheat (Table 3).

Possible role of the chromosomes

Based on cereal studies it seems that some of the genes controlling increased mineral stress tolerance (salt, Al, B, Mn, Cu) are localized on homologous group 5 (for review see Forster, 1992). In wheat this group plays an important role in the defence against other abiotic stresses such as low temperature (Sutka, 1981) and flooding (Poysa, 1984). Genes for frost resistance (*Fr-A1, Fr-B1, Fr-D1*) and vernalization requirement (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) were also physically mapped on these chromosomes (Galiba et al., 1995; Snape et al., 1997; Sutka et al., 1999, Tóth et al., 2003).

Table 4

Chromosomal localisation of genes for copper tolerance traits in wheat by screening Chinese Spring/Cappelle Desprez substitution series

Investigated trait:	Chromosome	Effect
Non-stress conditions		
Shoot length	6A	+ 7.8% *
Shoot dry weight	6A	+ 15.4% *
	1B	+ 13.0% *
Stress conditions		
Shoot length	5B	- 12.3% *
Tolerance parameters		
Relative shoot length	5A	- 11.9% **
	5B	- 7.1% *
	6B	+ 8.3% **
	5D	- 9.5% *
	7D	-10.7% **
Relative shoot dry weight	5A	-26.2% *
	6B	+ 15.0% *
	3D	+ 23.8% **
	5D	- 22.5% **
	7D	- 27.5% ***

*, **, ***: significant at the $P \leq 0.05$, 0.01 and 0.001, respectively. Chromosome substitutions 2A, 2B and 4D were not used for the experiment.

Interestingly, the genes for Cu tolerance in rye were physically mapped on chromosome 5R (Schlegel et al., 1993), and in the 5A/5RL wheat-rye translocation line the rye chromosome segment caused better Cu tolerance (Owuoche et al., 1996). It is possible that the uptake and/or translocation of copper could influence tolerance. Chromosome 5B plays a role in the defence against other abiotic stresses: genes controlling cadaverine biosynthesis may be localized on chromosome 5B (Galiba et al., 1993), and this chromosome also influenced hydroxamic acid accumulation in wheat (Niemeyer and Jerez, 1997). Cadaverine is a polyamine accumulating in plants under stress conditions, for example salt and osmotic stresses, and may contribute to proline accumulation (Bouchereau et al., 1999). Hydroxamic acids play a role as complexing agents in the uptake of iron by cereals (Pethő, 2002). The present study suggests that copper tolerance is under the control of more than one chromosome, indicating the polygenic character of Cu tolerance. The absence of any really Cu-tolerant wheat genotype suggests that there is no specific gene for Cu tolerance, but that genes responsible for defence against other abiotic stresses and for metal uptake may also play a role in Cu tolerance.

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ESTIMATION OF POLLEN VIABILITY IN EINKORN (*TRITICUM MONOCOCCUM* SSP. *MONOCOCCUM*) ACCESSIONS OF DIFFERENT GEOGRAPHICAL ORIGIN

G. KOVÁCS

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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In the present experiment the pollen viability of einkorn accessions of different origin was tested using four vital dyes (TTC, Baker's procedure, MTT and FDA) to determine the potential of the dyes to differentiate fresh living pollen from pollen heated for 12 hours at 80°C (killed pollen). It was found that two of the four dyes previously employed to determine pollen viability also stained killed pollen in the case of several einkorn accessions, while FDA and MTT did not. It is thus suggested that the two latter should be used to test einkorn pollen viability, since they do not normally stain either killed or aborted pollen grains.

Key words: *Triticum monococcum* ssp. *monococcum*, einkorn, drought tolerance, pollen viability, vital staining

Introduction

The ability to assess the pollen viability of einkorn accessions is very important in understanding and monitoring the effects of environmental stresses during the flowering period (Thomson et al., 1994). Pollen viability and performance are highly dependent on the environment, especially during the formation and maturation of male gametes. In this regard, environmental factors such as high temperature, drought conditions and soil fertility may seriously affect *in vivo* pollen performance (Zamir and Gaddish, 1987). Unfavourable climatic and environmental conditions are deleterious to pollen viability and can result in a decrease in fertility caused by complete pollen abortion. The consistent study of pollen viability and performance in einkorn accessions has several advantages. It can help to monitor negative changes in the environmental conditions, such as air and soil pollution (Bellani et al., 1988), the accumulation of toxic compounds, and various temperature and water stresses. On the other hand, pollen performance is a very good indicator of the abiotic stress tolerance of a given plant, helping in the selection of stress-tolerant genotypes.

Einkorn, which was the staple food of ancient societies and is one of the most promising donors of several abiotic stress tolerance genes for wheat breeding, generally flowers around two weeks later than hexaploid bread wheat, when high temperature and drought stress are very frequent under Hungarian growth conditions. Nowadays the original einkorn populations are in a critical situation in many places around Europe, as their traditional cultivation and

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in situ conservation is continuously decreasing. Besides declining human interest, the changing environmental conditions (e.g. pollution) are also dangerous for such important genetic resources. Although the measurement of the pollen viability of einkorn accessions will not defend the natural resources of these populations, the establishment of such monitoring systems could help in the maintenance of some still untouched populations in their original form. Unfortunately, to the best of our knowledge, no detailed studies have yet been carried out on the viability of einkorn pollen.

A large variety of dyes have been used to test pollen viability in several species, but only a few studies have tested the potential risk that such dyes will stain killed or aborted pollen grains (Rodriguez-Riano and Dafni, 2000). Since the most common vital dyes (Alexander's procedure, acetocarmine, aniline blue in lactophenol, TTC, MTT and X-Gal) have been strongly criticized, as they also stain killed pollen (Kapyla, 1991; Parfitt and Ganeshan, 1989; Khatum and Flowers, 1995; Sedgley and Harbard, 1993), the aim of the present study was to compare different vital dyes to determine their potential efficacy as indicators of einkorn pollen viability.

Materials and methods

Plant material

Pollen grains of four einkorn (*Triticum monococcum* ssp. *monococcum*) accessions of different ecological origin (MvGB 4, landrace, originating from the Carpathian Basin, MvGB 57, landrace, originating from Anatolia, MvGB 747, landrace, originating from Germany, and MvGB 1203, originating from Italy) were studied in the present experiment. Spikes of each accession were collected from the field at the time of anthesis and were brought into the laboratory. To collect the pollen grains from the spikes, a forced shedding method was used according to Pfahler et al. (1986). Depending on the amount of pollen, 2–3 spikes were used to study the effectiveness of the different viability tests. The freshly shed pollen from each spike was divided into two samples. The first sample was immediately stained with one of the vital stains, while the other was heated to 80°C for 12 hours (overnight). The second sample was designated as the dead pollen control. The whole experiment was replicated at least three times.

Pollen performance

The morphology of the mature einkorn pollen was studied using the procedure of Alexander (1969) in comparison with an unstained pollen population. For the unstained pollen studies the pollen grains were suspended in an appropriate concentration of sucrose. The number of normally developed, young and aborted pollen grains was counted separately.

Viability staining tests

Four different staining methods were used to test pollen viability.

a, Tetrazolium test

The tetrazolium test (Aslam et al. 1964) is based on the reduction of a colourless soluble tetrazolium salt to a reddish insoluble substance called formazan in the presence of dehydrogenases. Nitroblue tetrazolium and 2,3,5-triphenyl tetrazolium (TTC) are the most commonly used tetrazolium salts. The test solution consisted of 0.2–0.5% TTC in 0.1 M phosphate buffer (pH = 7.2) with the concentration of sucrose required to prevent the pollen grains from bursting. Pollen grains were considered viable if they turned red.

b, Baker's procedure

Baker's procedure (Dafni, 1992) detects the presence of alcohol dehydrogenase. The test solution consisted of 7 mg phosphate buffer/10 ml water (pH 7.3), 6 mg nitroblue-tetrazolium, 6 mg nicotinamide adenine dinucleotide and 0.5 ml ethanol (35%). Pollen grains were considered viable if they turned violet or pink.

c, MTT test

The MTT test (Norton, 1966; Khatum and Flowers, 1995) detects the presence of dehydrogenase. The test solution consisted of a 1% concentration of the substrate 2,5-diphenyl tetrazolium bromide (MTT) or thiazol blue in 5% sucrose. Pollen grains were considered viable if they turned deep pink or if they showed no colour but had irregular black lines on the surface (according to Rodriguez-Riano and Dafni, 2000).

d, Fluorochromatic reaction (FCR) test

When pollen grains are mounted in fluorescein diacetate (FDA) solution, the non-polar, non-fluorescent FDA readily enters the pollen cytoplasm. Cytoplasmic esterases hydrolyse FDA and release fluorescein, which is polar and fluorescent (Heslop-Harrison et al., 1984). Unlike FDA, fluorescein passes sparingly through an intact membrane and therefore accumulates in the cytoplasm of viable pollen grains and gives a bright green or yellowish green fluorescence under the fluorescence microscope. For testing pollen viability fluorescein diacetate was dissolved in acetone (5 mg/ml) and used at 10^{-6} M in 60% (w/v) sucrose according to Heslop-Harrison and Heslop-Harrison (1970). The pollen was considered viable if strong bright fluorescence was observed under the fluorescence microscope (490–510 nm).

All non-fluorescent pollen viability tests were conducted by incubating the pollen in the medium for at least 30 min at 35°C. Three replications each sample and three random groups of at least 300 pollen grains per replication were counted in the present experiment. In the case of the FDA test, a similar number of pollen grains were studied 1–3 minutes after staining.

Statistical analysis was carried out using the SPSS for Windows 10.0 statistical software package.

Results and discussion

Einkorn pollen grains are relatively small compared with other cereal species and the majority of freshly shed pollen has very good performance. According to pollen morphological studies (Table 1) the majority (up to 95%) of the pollen grains of the different einkorn samples showed normal morphological features, and only the pollen population of the Italian landrace contained a significant amount of young, still vacuolated immature microspores. Interestingly enough, very few aborted or sterile pollen grains were observed under light microscopy without staining in any of the samples, while the percentage of abnormal pollen grains increased after staining. The cytoplasm ripeness test (Alexander's test) is very sensitive to the osmotic potential of the dye, so several pollen grains burst, and the increased ratio of aborted or sterile pollen grains was a consequence of staining. The results suggest that to obtain realistic data on the pollen performance of the different einkorn populations it is better to avoid staining. Comparing the pollen performance of the four einkorn accessions studied, the MvGB 4 landrace, originating from the Carpathian Basin, showed the best performance under Hungarian ecological conditions, followed by accession MvGB 57, of German origin. Relatively poorer results were obtained for the landrace from Anatolia (MvGB 747), while the percentage of normally matured pollen in the Mediterranean landrace (MvGB 1203) was significantly lower than that of MvGB 4. The results of the pollen performance studies suggest that accessions originating from continental climates are well adapted to Hungarian growth conditions, while such conditions are unfavourable to populations originating from the Mediterranean.

The cytoplasm ripeness test (Alexander's test) completely stained the killed einkorn pollen grains as well, so it cannot be used as a viability dye. The temperature treatment used to kill the pollen grains did not modify the pollen morphology or staining significantly (data not shown).

According to the results of the present experiment, the einkorn pollen viability rates obtained fluctuated depending on the test used. The maximum values were obtained with the TTC test in most cases, lower but similar values were given by Baker's procedure and the MTT method, and the lowest viability percentages were given by the FDA test (Table 2).

The results of the experiment indicate that there are three groups of dyes: those that always stained killed pollen in the case of einkorn, those that stained killed pollen in some replications but not consistently across the experiment, and those that never stained killed pollen. Dyes in the first group (TTC and Baker's) always stained some killed pollen grains. The difference between these two dyes was that Baker's stained killed pollen faster than fresh pollen, while TTC stained killed pollen at the same rate as fresh pollen. With the TTC staining solution, where 80% of pollen stained red even after killing by heating, the reduction was presumably brought about by some heat-stable component of the pollen. Whatever the mechanism of the reduction, the use of TTC is obviously not appropriate for testing the viability of einkorn pollen. Similar results were obtained by Rodriguez-Riano and Dafni (2000) in the case of Baker's procedure. It is thus recommended to avoid the use of TTC and Baker's test to study the pollen viability of einkorn.

Table 1

Average morphological features of freshly shed pollen grains from different einkorn accessions

Einkorn accession	Treatment	% of mature pollen	% of immature pollen	% of sterile or abnormal pollen
MvGB 4	Without staining	99.0	1.0	0.0
	Alexander's test	97.2	0.8	2.0*
MvGB 57	Without staining	98.1	0.9	1.0
	Alexander's test	97.3	0.8	1.1
MvGB 747	Without staining	97.9	2.1	0.0
	Alexander's test	97.5	1.7	0.8*
MvGB 1203	Without staining	96.5	2.3	1.2
	Alexander's test	94.3	2.0	3.7*

* Significantly different from the unstained control at the p = 0.05 probability level.

Table 2
Viability percentage of fresh and killed pollen in different einkorn accessions as determined by four vital dyes

Einkorn accession	Test	Fresh	Killed
MvGB 4	TTC	87.6	85.4
	Baker's	83.6	81.7^{ns}
	MTT	82.1	0.0**
	FDA	81.1	0.0***
MvGB 57	TTC	82.5	88.4
	Baker's	84.6	70.2^{ns}
	MTT	81.7	1.7***
	FDA	79.8*	0.0***
MvGB 747	TTC	72.6	73.4
	Baker's	83.6	71.7^{ns}
	MTT	55.1**	1.2***
	FDA	58.1**	0.0***
MvGB 1203	TTC	85.6	85.4
	Baker's	84.6	81.7^{ns}
	MTT	61.1**	0.0***
	FDA	68.3**	0.0***

ns - not significant, *,**,*** - significant at the p= 0.05, 0.01and 0.001 probability levels, respectively

The second group (MTT) showed many different colour tonalities and sometimes the very dark pink pollen was difficult to distinguish from the black. In addition, MTT seldom stained killed pollen, although when it did the stain was always lighter than with fresh pollen. Therefore, MTT should be used with caution, taking into consideration the sensitivity of the species and genotypes being tested. Parfitt and Ganeshan (1989) found that, in the case of some *Prunus* species, heat-killed pollen was intensely stained, and Rodriguez-Riano and Dafni (2000) also found that pollen heat-treated for two hours was sometimes stained in several species.

The use of the fluorescein diacetate test was the most reliable method to distinguish between fresh and killed pollen, since dead pollen were completely black (transparent) or only the pollen wall showed limited fluorescence under UV light. Within 2–3 minutes of incubating fresh pollen in fluorescein diacetate live pollen grains appeared bright green. Unfortunately, the fluorescence of viable pollen grains changes intensively over time. During counting, some of the initially bright green pollen grains showed a decrease in fluorescence and after 5 minutes the pollen population became highly variable. After five minutes some pollen grains still fluoresced very brightly while others were already non-fluorescent. This suggests that einkorn pollen is highly sensitive to UV light, which gradually kills the pollen grains. Probably there is a variability in the UV light tolerance of the pollen populations. This suggests that great care must be taken when using this method to monitor the pollen viability of einkorn.

Out of the four dyes tested, only the MTT and FDA tests showed a high correlation with pollen viability, as they did not stain either killed or aborted pollen. The variability of colour tones and the time-dependent decrease in fluorescence may also make it difficult to differentiate between fresh and killed pollen. The prompt use of FDA is probably the most promising method to solve this problem.

Based on the results of the present study, it is recommended that some type of control (such as killed pollen) should be used to check the potential of the method to test pollen viability before using it. If it stains killed pollen, then it must be avoided, as also stated by Rodriguez-Riano and Dafni (2000). The next step should be to test the potential capability of the dye to stain non-germinated pollen at different ages, because sometimes the use of vital dyes is unsatisfactory (Sedgley and Harbard, 1993). The present results suggest that the MTT or FDA tests should be used to monitor pollen viability in einkorn. The use of these vital dyes could also help to follow environmentally induced changes in einkorn populations.

No significant difference in pollen viability was obtained between the different populations in the case of the TTC test or Baker's procedure. By contrast a significant decrease in pollen viability was detected by FDA method in the case of MvGB 747 and MvGB 1203 compared with the continental types MvGB 4 and MvGB 57. These results suggest that the study of pollen viability in einkorn could be successfully used to investigate the adaptability and environmental stress tolerance of einkorn.

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GRADIENT CHAMBER STUDIES ON THE DRY MATTER ACCUMULATION OF WINTER EMMER [*TRITICUM TURGIDUM* SSP. *DICOCCON* (SCHRANK) THELL.] LANDRACES IN THE SEEDLING STAGE

B. KŐSZEGI and G. KOVÁCS

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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The multiplication and characterisation of genetic stocks originating under very different ecological conditions is a problem constantly encountered in gene bank research. However, the major components of the original environment, such as temperature, light and humidity, can be reproduced under artificial conditions in the phytotron. The gradient, or inhomogeneous, chamber available in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, makes it possible to elaborate plant growth programmes optimised for the various developmental phases of each population in a single step. In this chamber gradients of two extremely important environmental factors, temperature and illumination, can be simultaneously programmed, thus allowing the optimum light × heat combinations to be identified. However, the use of complete inhomogeneity (light × heat) makes it extremely difficult to evaluate the experimental results, since biometric methods based on traditional statistics are unable to handle this situation. It is thus essential to find a method suitable for the comparative analysis of continual variables (Okada et al., 2000).

The present paper reports on the first phase in the development of a plant growth programme for emmer, based on investigations made on two gene bank accessions of winter *Triticum turgidum* ssp. *dicoccon* (Schrank) Thell. (MvGB 301 and MvGB 304). In the gradient chamber study the accumulation of dry biomass in three-week-old plants was investigated as a function of temperature and light intensity.

The results suggest that a temperature of 10–12°C combined with low or moderate light intensity is optimum for the germination and initial development (0–4 weeks) of emmer. These conditions also induced good tillering, which is extremely important, especially for gene bank accessions where the possibility of seed multiplication and field cultivation is limited.

Key words: emmer wheat, gradient plant growth chamber, initial development, climatic programme, dry matter accumulation

Introduction

One problem regularly faced by gene bank scientists is how to multiply and characterise valuable genetic stocks originating from locations with a completely different environment. The research initiated on emmer [*Triticum turgidum* ssp. *dicoccon* (Schrank) Thell.] in recent years has often been hindered by the fact that it proved impossible to multiply valuable germplasm obtained from foreign gene banks either in the field or under artificial plant growth conditions. The climatic conditions in Hungary were alien to these populations, and the stress suffered in various stages of development in the field, together

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with attacks by pathogens they had not previously encountered, put such a strain on the plants that the majority of them died, while those that remained were mostly sterile. Similar problems arose during plant growth in the phytotron. The cereal growth programmes developed in the Martonvásár phytotron, based on many years of experience, were optimised for winter wheat (Tischner et al., 1997) and attempts to raise emmer accessions on these programmes were generally unsuccessful. However, if a programme is to be elaborated for the cultivation of "exotic" emmer populations, relatively large quantities and a series of experiments carried out in several differently programmed growth chambers will be required, involving substantial costs and time.

The gradient, or inhomogeneous, chamber, however, makes it possible to compose optimum plant growth programmes for the various developmental stages of emmer populations in a single step, since relatively wide gradients can be programmed for two extremely important environmental factors (Tischner and Veisz, 1996). A temperature gradient can be set up in one direction on the growth bench, while a light intensity gradient can be programmed perpendicularly to this in such a way that each of the 12 × 12 plants arranged in the rows and columns is grown in a different microecological environment. In this way the optimum light × heat combination for each developmental phase can be relatively easily identified by scoring and measuring the plants, so in theory a single generation should be sufficient to elaborate an optimum plant growth programme. The use of complete inhomogeneity, however, causes serious difficulties when evaluating the experimental results, since biometric methods based on traditional statistics were not designed for this situation (Okada et al., 2000). For this reason, earlier experiments in the gradient chamber tended to use only one of the gradients, to ensure that the replications required for statistical evaluation could be set up (Berzsenyi and Győrffy, 1989; Marton, 1990; 1991; Nagy and Berzsenyi, 1984). These experiments confirmed that the genotype × temperature and genotype × temperature × chemicals interactions could be adequately evaluated in the gradient chamber (Tischner and Veisz, 1996).

The present experiments in the gradient chamber were designed as the first stages in the development of a plant growth programme for emmer, and were based on emmer gene bank accessions of various origins. This paper describes the accumulation of dry biomass in three-week-old plants as a function of temperature and light intensity.

Materials and methods

Two winter landraces of *Triticum turgidum* ssp. *dicoccon* (Schrank) Thell. of different origin (gene bank accessions MvGB 301 and MvGB 304) were used in the experiments. MvGB 301 evolved on the eastern side of the Ukrainian Carpathians and MvGB 304 on the western side of the Caucasian mountains. Both are true winter types which do not head without vernalisation. Eighty spikelets from each accession were chosen for the initial development studies. The grains from these spikelets were soaked for 12 hours, without dehulling, at 20°C, after which they were planted in pots, according to the procedure usual in phytotronic plant raising (Tischner et al., 1997). The pots were then placed in the gradient chamber (Fig. 1) with alternate rows of each variety (Tischner and Veisz, 1996). The emergence and initial development of the two emmer landraces were investigated using a cross-gradient, where the temperature in the 12 rows ranged from 8°C to 18°C (Fig. 2/a), while the light intensity in the 12 columns covered a range of 210–540 μmol m^{-2} s^{-1} (Fig. 2/b). A 16-h day was programmed throughout the experiment. An environmental index was calculated for the real values of the cross-gradient, plotted in Figure 3 for the whole of the bench area.

It is clear from Figure 2 that all the positions on the growth bench represented a different environment for the plants. However, this completely inhomogeneous system made it impossible to regulate the relative humidity of the air. Irrigation was carried out manually to provide the optimum moisture content for each plant, since the varying environmental conditions resulted in differing levels of water consumption and transpiration as a function of the temperature.

The plants were grown under the same conditions for three weeks, after which the plants were cut off at ground level for the determination of fresh and dry mass (to the nearest mg). The results were evaluated statistically using the SPSS 8.0 programme package.

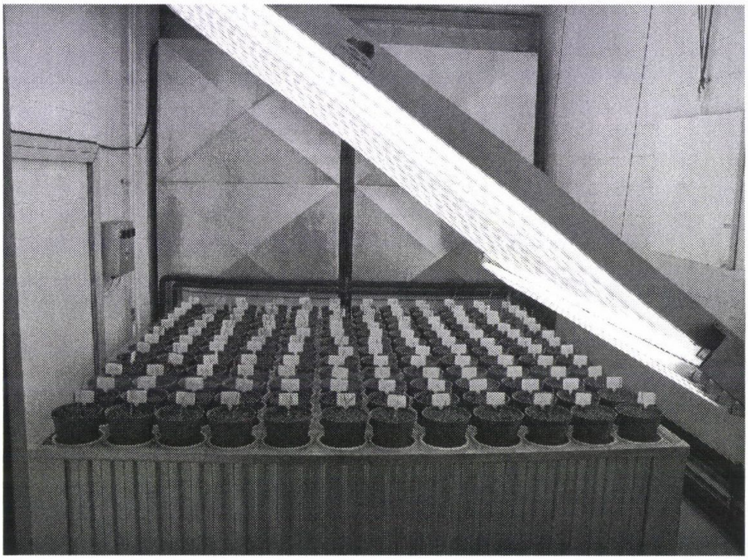

Fig. 1. The gradient chamber

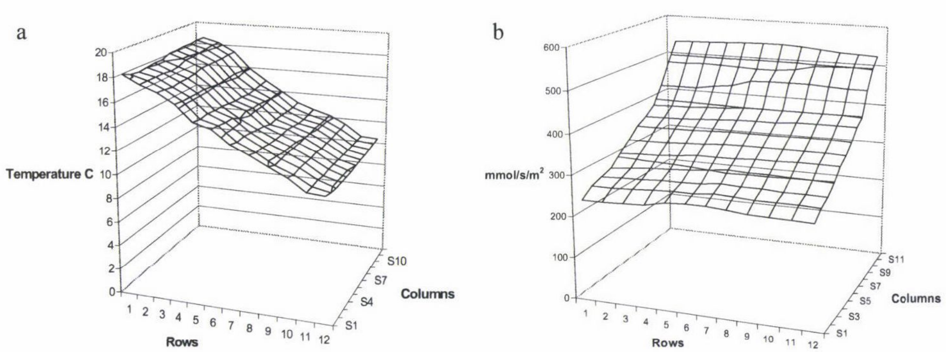

Fig. 2. The temperature (a) and light (b) gradients programmed in the gradient chamber

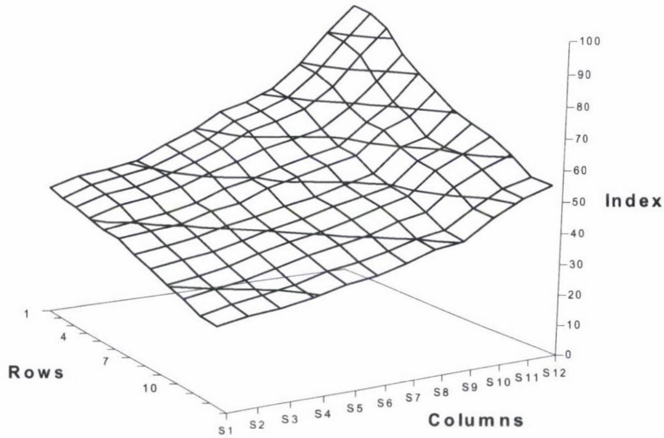

Fig. 3. Distribution of temperature and light intensity in the gradient chamber

Results and discussion

The experimental results make it quite clear that the dry matter accumulation of emmer in the initial phases of development was influenced to a far greater extent by the temperature than by the light intensity. Changes in light intensity had practically no effect in the low temperature range, while even a slight rise in the temperature led to a significant increase in biomass production. As the temperature rose, the importance of light intensity gradually increased, and at relatively high temperatures it had a stimulating effect on biomass production. This is clearly illustrated by the figure illustrating the effect of the highest temperature, where the dry matter production was only half as great at low light intensity as it was in plants grown at the same temperature with high light intensity (Fig. 4). The overall picture shows, however, that the positive effect of light intensity was only manifested at temperatures above 15°C. Averaged over the whole experiment, the positive effect of temperature and light gradients on dry matter production could be expressed by a linear model (Fig. 5). For both the gradients the regression lines give a good fit to the measured values, indicating that the use of a linear model is adequate. The magnitude of the b values obtained for the linear regression model (b = 0.0094, R^2 = 0.9781) confirms that the temperature gradient had a far greater effect on dry matter accumulation than the light intensity levels applied (b = 0.0037, R^2 = 0.8482).

A comparison of the two emmer genotypes indicated that there was practically no difference between them with respect to dry matter production during initial development. In the low temperature range no difference could be demonstrated between the two genotypes (Fig. 6), nor was there any real

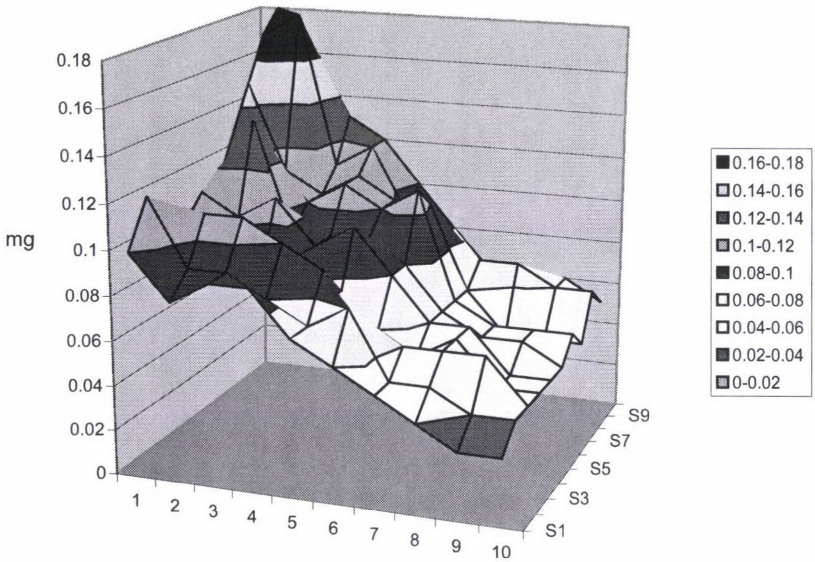

Fig.4. Effect of temperature and light intensity gradients on the dry matter production ability of emmer varieties

difference between the temperature-dependent regression lines of MvGB 301 ($b_1 = 0.0102$) and MvGB 304 ($b_2 = 0.0100$), while the significance of the model was extremely high ($R^2_1 = 0.9631$; $R^2_2 = 0.9645$). The situation was similar for the light gradient, except that MvGB 301 exhibited far greater variability in the low light intensity range than MvGB 304. The steepness of the regression lines for the two varieties was almost identical ($b_1 = 0.0043$; $b_2 = 0.0046$), but the values were only about half those recorded as a function of temperature. The regression coefficient was also significantly lower than that obtained for the temperature gradient.

The results make it quite clear that when compiling a plant growth programme for emmer, the correct choice of temperature is far more important than that of the light intensity. The data also indicate that at the <15°C temperatures required for tillering, the light intensity plays very little role in determining the level of biomass production. Since low intensity illumination is sufficient for satisfactory initial development even at relatively high temperatures, the use of low light intensity could help to reduce costs when elaborating a plant growth programme for emmer. As significant differences were not observed in the biomass accumulation of emmer genotypes originating from ecologically differing environments, there appears to be a good chance of developing a cost-saving plant growth programme which can be applied for a wide range of genotypes.

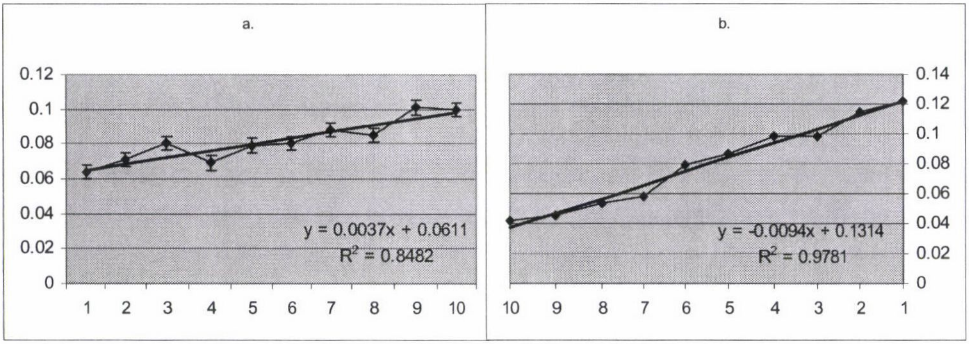

Fig. 5. Effect of light intensity (a) and temperature (b) on dry matter accumulation

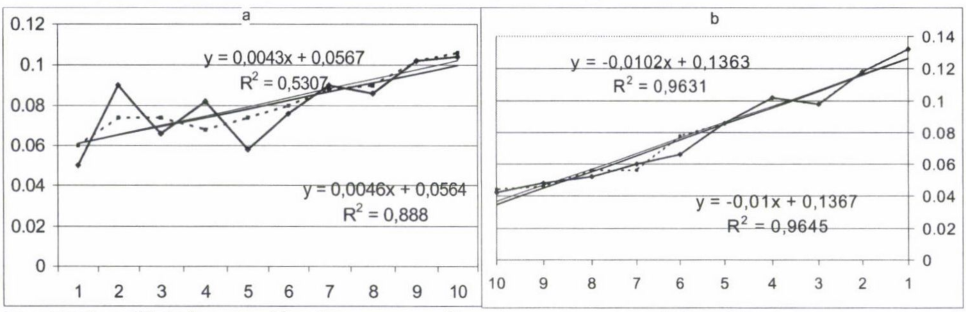

Fig. 6. Effect of light intensity (a) and temperature (b) on dry matter accumulation of the two emmer genotypes

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IDENTIFICATION OF QTLs INVOLVED IN PHYSIOLOGICAL AND AGRONOMIC INDICATORS OF DROUGHT TOLERANCE IN RYE USING A MULTIPLE SELECTION INDEX

E. FARSHADFAR[1], R. MOHAMMADI[1], M. AGHAEE[2] and J. SUTKA[3]

[1]COLLEGE OF AGRICULTURE, RAZI UNIVERSITY, KERMANSHAH, IRAN
[2]DRYLAND AGRICULTURAL RESEARCH INSTITUTE, SARAROOD, KERMANSHAH, IRAN
[3]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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Water deficiency is a major constraint in wheat production and the most important contributor to yield reduction in the semiarid regions of the world. Species related to wheat are valuable genetic sources for different traits including resistance/tolerance to biotic and abiotic stresses. To locate the genes controlling the physiological and agronomic criteria of drought tolerance, disomic addition lines of *Secale cereale* cv. Imperial (donor) into the genetic background of *Triticum aestivum* cv. Chinese Spring (recipient) were tested under field, greenhouse and laboratory conditions. Disomic addition lines exhibited significant differences for relative water content (RWC), relative water loss (RWL), water use efficiency (WUE) and stomatal resistance (SR), indicating the presence of genetic variation and the possibility of selection for improving drought tolerance. Three physiological variables, RWL, WUE and SR, with high correlation with the stress tolerance index (STI) and germination stress index (GSI), contributed 69.7% to the variability of yield under stress (Ys) in the regression equation. Based on the physiological multiple selection index (MSI) most of the QTLs controlling physiological indices of drought tolerance were located on chromosomes 3R, 5R and 7R. The contribution of addition line 7R to the MSI was 47%. The evaluation of disomic addition lines for STI and GSI revealed that most of the QTLs involved in these quantitative criteria of drought tolerance are located on 3R and 7R. Cluster analysis and three dimensional plots of Ys, yield potential (Yp) and MSI indicated that 3R and 7R are the most important chromosomes carrying useful genes for improving drought tolerance.

Key words: *Secale cereale,* disomic addition lines, stress tolerance index, germination stress index, physiological multiple selection index

Introduction

Water deficiency is a major constraint in wheat production and the most important contributor to yield reduction in semiarid regions (Ehdaie and Waines, 1993; Kristin et al., 1997; Andrew et al., 2000). Improving drought-resistant cultivars is, therefore, a major objective in plant breeding programmes for rainfed agriculture in these regions (Ehdaie et al., 1991; Ehdaie and Waines, 1993).

To understand the genetics of continuous variation, it is necessary to identify the chromosomal location of the genes controlling quantitative attributes (Eskridge et al., 2000). Identification of the genetic architecture of a quantitative character such as drought is a prerequisite for finding out the genetic bases of heterosis, selection in appropriate generations and the production of pure lines

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and hybrids in a plant breeding programme. For these reasons the genetic analysis of quantitative characters has been conducted using biometrical, cytological and molecular methods (Farshadfar, 1998; Morgan, 1991).

Various quantitative traits (including morphological and physiological characteristics) have been proposed for the selection of resistant/tolerant genotypes to drought stress (Passioura, 1983; Fernandez, 1992; Kristin et al., 1997). Physiological attributes such as relative water content (RWC), chlorophyll fluorescence (CHF), proline accumulation, abscisic acid accumulation (ABA), osmotic adjustment, root size, stomatal resistance (SR), CO_2 exchange and water use efficiency (WUE) (Blum, 1988; Al-Dakheel, 1991; Loss and Siddique, 1994) are associated with drought stress tolerance/resistance.

Most of the wide hybridization studies reported have been performed to transfer major genes for resistance to biotic stresses like disease (Sharma and Gill, 1984; Gale and Miller, 1987; Knott, 1987; Islam and Shepherd, 1991; Jiang et al., 1994). Not much work has been done on the transfer of quantitative traits such as drought, cold and salinity tolerance. This is mainly because of the fact that these traits are mainly governed by minor genes with small effects (QTLs).

Because of the complex nature of drought tolerance, little information is available on the chromosomal location of the genes conditioning drought tolerance and related physiological traits affecting drought tolerance (Farshadfar, 1995).

Species related to wheat, including both distantly related and progenitor species, represent a large reservoir of useful variability that can be exploited in wheat improvement (Jiang et al., 1994; Friebe et al., 1996). They contain indispensable genes required for wheat improvement especially under an unfavourable environment. They generally have tolerance to biotic stresses and survive under low input conditions. Disomic alien addition lines (DAALs), in which single pairs of homologous chromosomes from a related species are added to the wheat complement, are worthwhile material to identify alien chromosomes carrying useful genes and form the starting point for the cytogenetic transfer of alien genetic material to wheat (Gale and Miller, 1987). Several addition lines have been produced in wheat with improved quality for disease resistance, earliness, winter hardiness and protein content. However, none of them have been accepted as commercial varieties (Khush, 1973).

The present investigation was therefore carried out to screen the physiological criteria of drought tolerance and to identify the chromosome(s) most probably carrying physiological traits affecting drought tolerance in rye.

Materials and methods

To locate QTLs controlling field and laboratory predictors of drought tolerance, disomic chromosome addition lines of *Secale cereale* cv. Imperial (2n=2x=14) into the genetic background of Chinese Spring (CS) wheat (2n=6x=42) and two checks, *Triticum aestivum* L. cv. Sardary, a drought-tolerant land race from west Iran, and a rye variety, Lovászpatonai, were used in various field and laboratory experiments. In the field, each line was sown in 2 rows 120 cm in length with 20 cm row to row and 3 cm plant to plant spacing under two water regimes (stressed and non-stressed) using a randomized complete block design (RCBD) with three replications. Besides yield potential (Yp) and stress yield (Ys) the following physiological criteria were measured:

1. Relative water content (RWC): A sample of five leaves was taken randomly from each line and fresh weight (FW) was measured with a digital balance. The leaf samples were oven dried at 70°C for 72 h and weighed (dried weight=DW). RWC was then calculated using the formula of Alidib et al. (1990):

$$\%RWC = [(FW - DW) / FW] \times 100$$

2. Relative water loss (RWL): Five leaves from each line were collected and weighed. The leaves were then wilted at 30°C and reweighed, transferred to the oven for 24 h and weighed again. RWL was calculated using the formula suggested by Yang et al. (1991):

$$RWL = (W_1 - W_2 / W_3)\,(t_1 - t_2 / 60)$$

where W_1, W_2 and W_3 are the initial, wilted and dried weights, and t_1 and t_2 are the time of measurement for initial and wilted weight (in minutes).

3. Chlorophyll fluorescence (CHF): From each line in each replication 5 flag leaves were selected and the quantum yield was recorded after dark adaption using a MINI-PAM instrument as:

$$\text{Quantum yield} = F_v / F_m$$

where F_v and F_m are variable and maximum fluorescence, respectively (Gently et al., 1989).

4. Stomatal resistance (SR): Five random flag leaves were selected from each line and SR was measured in s cm^{-1} by Prometer. If the instrument is calibrated in cm s^{-1}, the stomatal conductivity can be measured, which is the inverse of stomatal resistance.

5. Water use efficiency (WUE): To measure WUE, the genotypes were compared in a completely randomized design (CRD) with three replications. Three seeds from each line were sown in the greenhouse, two of which were eliminated 10 days after germination. To calculate the amount of evaporation, one empty pot was used in each replication. The pots were irrigated with the measured amount of water. The run-off water in each pot was subtracted from the water applied to each pot. After 39 days, the dry matter (after drying at 70°C for 24 h) and the amount of water applied were used to calculate WUE using the formula suggested by Ehdaie and Waines (1993):

$$WUE = DM / WU$$

where DM and WU are dry matter and water used, respectively.

6. Germination stress index (GSI): In the laboratory experiment 50 seeds from each disomic addition line were germinated on wet filter paper in Petri dishes, after which the Petri dishes were arranged in a completely randomized design with three replications under two different stress and non-stress water regimes in the growth chamber. Day and night temperatures were 20°C and 15°C, respectively, and the relative humidity was 75%. To create stress and non-stress conditions 10 ml of PEG with –0.8 MPa osmotic potential and 10 ml of distilled water were used in the Petri dishes, respectively. After 10 days the number of germinated seeds was recorded, and the promptness index (PI) and germination stress index (GSI) were calculated using the formula given by Bouslama and Schapaugh (1984):

$$PI = nd_2\,(1.0) + nd_4\,(0.8) + nd_6\,(0.6) + nd_8\,(0.4) + nd_{10}\,(0.2)$$

where nd_2, nd_4, nd_6, nd_8 and nd_{10} are the percentage of germinated seeds on the 2^{nd}, 4^{th} , 6^{th} , 8^{th} and 10^{th} day, respectively.

$$GSI = [(\text{PI, under stress conditions})/(\text{PI, under non-stress conditions})] \times 100$$

7. Two different experiments were carried out in the field under irrigated and water stress conditions. The seeds were sown in 1 m rows with 3×25 cm plant to plant and row to row distances, respectively. The stress tolerance index (STI) was calculated from yield potential (Yp) and stress yield (Ys) by the formula suggested by Fernandez (1992):

$$STI = (Ys)\,(Yp) / (\overline{Yp})^2$$

where $\overline{Yp}$ is the overall mean of the entries under non-stress conditions.

8. Multiple selection index (MSI): The value of each physiological trait (RWC, RWL, CHF, SR and WUE) was first standardized for each line, after which the MSI was calculated as:

$$MSI = RWC_{std} + RWL_{std} + CHF_{std} + SR_{std} + WUE_{std}$$

9. Efficiency of the added chromosome (EAC): The EAC for each line was calculated after eliminating the MSI of the recipient (Chinese Spring = CS) as:

$$EAC = \frac{\text{MSI of addition line} - \text{MSI of CS}}{\text{MSI of CS}} \times 100$$

Analysis of the data was performed using the statistical packages MSTAT-C, SPSS and HWG. Cluster analysis of the genotypes for STI and MSI was done using the UPGMA procedure.

Results

The analysis of variance showed highly significant differences between the genotypes for all the physiological traits except CHF (Table 1).

Mean comparison also revealed the presence of different groups of genotypes (Table 2), indicating the presence of genetic variability for the traits under investigation.

The stomatal resistance was high for 7R, 5R, 3R, the donor parent and the check, so these were placed in a single group (Table 2). The check (Sardari) showed the highest WUE. Among the addition lines (Table 2), the yield under stress conditions was higher for 7R and 3R, and low for 4R, 2R and 6R. On the other hand, lines 3R, 5R, 7R, 1R and the check produced more yield under non-stress conditions (Table 2).

The mean performance of RWC ranged from 69.5% for addition line 4R to 175.8% for 6R, which was significantly different from the rye checks (Imperial and Lovászpatonai). RWL ranged from 0.013% for addition line 7R to 0.45 for 2R.

No significant difference was observed between the genotypes for quantum yield, measured by CHF (Table 2). However, the CHF value was higher for addition lines 3R and 5R, and for the donor parent.

Germination analysis showed the highest values of GSI for addition lines 7R and 3R and the check, while it was low for lines 2R and 4R.

Correlation analysis revealed a positive, significant relationship between RWC and both WUE (r=0.5258**) and MSI (r=0.743**) (Table 3).

Table 1
Analysis of variance and mean squares of physiological traits

Source of variation	D.F.	Mean squares				
		RWC	RWL	WUE ^	SR	CHF
Replication	2	183.58**	0.000006	–	0.869	0.002
Genotype	10	2151.83**	0.0003**	0.002**	7.92**	0.002
Error	22	45.79	0.0000007	0.000061	0.979	0.001
CV%		5.84	8.65	5.46	15.0	4.47

^ WUE was compared in a CRD design; ** Significant at the 0.01 level of probability.

Table 2
Mean comparison of traits and drought tolerance criteria in disomic addition lines and the checks

Genotype	RWC%	RWL	CHF	SR	WUE	Ys	Yp	STI*	GSI*	MSI*	EAC*%
1R	87.5d+	0.26ab	0.811a	7.16ab	0.136d	56.70bcd	87.40abc	0.749	60.29	10.76	12
2R	100.1cd	0.45a	0.778a	4.02c	0.096g	17.55ef	49.56c	0.121	36.66	2.44	–74
3R	126.2d	0.28ab	0.826a	7.89ab	0.110f	73.92abc	89.65ab	0.991	61.80	11.45	19.5
4R	69.5e	0.045a	0.778a	4.51c	0.101g	11.07f	62.61bc	0.096	34.66	4.00	–42
5R	113.4bc	0.031ab	0.830a	8.43a	0.111f	62.79abcd	84.49abc	0.789	60.01	11.39	19
6R	175.8a	0.048a	0.822a	5.70bc	0.131e	34.93def	77.60abc	0.426	50.79	10.09	5
7R	121.5b	0.013b	0.797a	8.85a	0.142d	80.97ab	101.9ab	1.223	65.71	13.57	42
Imp.	126.3b	0.03ab	0.826a	7.76ab	0.158b	60.73abcd	78.18abc	0.714	62.29	14.71	–
Lov.	126.6b	0.035a	0.818a	5.45bc	0.147c	44.34cdf	75.34bc	0.490	52.27	10.53	–
CS	115.4bc	0.028ab	0.800a	5.55bc	0.131e	56.70bcd	77.77abc	0.485	62.20	9.58	1
Sardari	110.7bc	0.027ab	0.785a	7.20ab	0.167a	88.22a	121.1a	1.578	88.19	11.51	–

+ Figures with the same letter were not significant at the 5% level of probability; *No analysis of variance was performed; Imp.: Imperial, Lov.: Lovászpatonai

Table 3
Phenotypic correlation coefficients between the traits under investigation

Traits	CHF	RWC	RWL	SR	STI	GSI	YP	YS	WUE	MSI
CHF	1.0									
RWC	0.528	1.0								
RWL	–0.108	–0.213	1.0							
SR	0.474	0.469	–0.794**	1.0						
STI	0.071	0.376	–0.768**	0.792**	1.0					
GSI	0.145	0.448	–0.712*	0.689**	0.932**	1.0				
YP	0.002	0.485	–0.712*	0.725*	0.966**	0.936**	1.0			
YS	0.247	0.859**	–0.859**	0.855**	0.949**	0.937**	0.905*	1.0		
WUE	0.266	0.526**	–0.489	0.406	0.628*	0.752**	0.680*	0.621*	1.0	
MSI	0.646*	0.743**	–0.707*	0.854**	0.716**	0.746**	0.708*	0.826**	0.730*	1.0

*, ** significant at the 5% and 1% level of probability, respectively

A negative, significant correlation was observed between RWL and SR (r= –0.794**), GSI (r = –0.712*), Ys (r = –0.859**) and MSI (r = –0.707*).

STI showed a high positive correlation with both Ys (r=0.826**) and Yp (r=0.708*). The correlation of MSI with STI and GSI was positive and significant (Table 3). Among the physiological traits, RWL, SR and WUE had a significant correlation with STI and GSI.

Regression analysis was performed considering Ys as a dependent variable and RWC, RWL WUE, SR and CHF as independent variables (Table 4).

Table 4
Traits entered in the equation of stepwise regression for yield under stress

S.O.V	B	SE B	T	Sig. T
RWL	-991.9224	333.4447	-2.975**	0.0059
SR	5.5201	1.8189	3.035**	0.0050
WUE	276.8312	120.6360	2.295*	0.0292

The final regression equation using the stepwise selection procedure was as follows:

$Y_S = 13.13 - 0.92\ \mathrm{RWL} + 5.52\ \mathrm{SR} + 276.83\ \mathrm{WUE}$

Multiple R = 0.85148

$R^2 = 0.725$

$R^2_{(Adj)}=0.697$

The three variables, RWL, SR and WUE, which were highly correlated with STI and GSI, determined about 69.7% of variability, as indicated by the adjusted R^2 ($R^2_{(Adj)}=0.697$). Selection efficiency was calculated as the MSI of each line as a percentage of the MSI of the recipient parent (Table 2). This criteria showed that the MSI of addition lines 7R, 3R and 5R increased the MSI by 42%, 19.5% and 19%, respectively. On the other hand, the addition lines 2R and 4R decreased MSI by 74% and 42%, respectively.

Cluster analysis of the genotypes was performed based on STI and MSI. Discriminant analysis of the clusters grouped the genotypes into three different classes. The first group included the addition lines 3R, 5R, 1R and 6R, the donor parent and the recipient. The second group included 7R and the check, while 2R and 4R formed the third group (Fig. 1).

A three-dimensional representation of Ys, Yp and MSI is shown in Figure 2.

The area of the 3D plot was divided into 4 regions, a, b, c and d (Fernandez, 1992). Lines 7R, 3R and 1R, along with the check (Sardari) were placed in the a region of the plot, which had the highest MSI, Ys and Yp (Fig. 2).

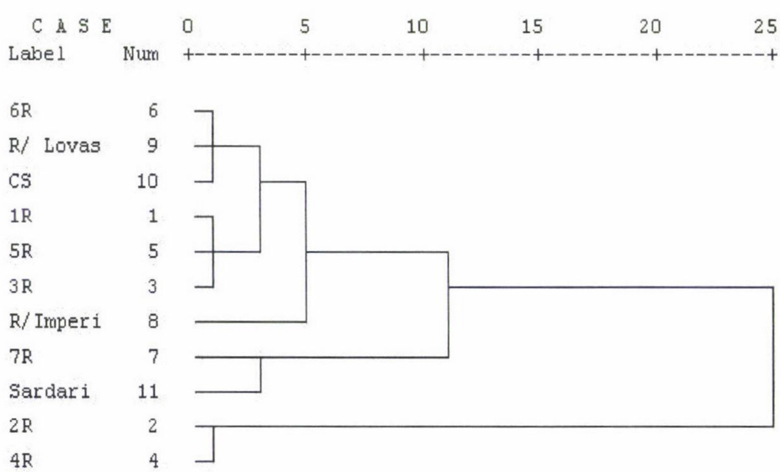

Fig. 1. Dendrogram resulting from cluster analysis on disomic additions based on STI and MSI (Lovas: Lovászpatonai, Imperi: Imperial)

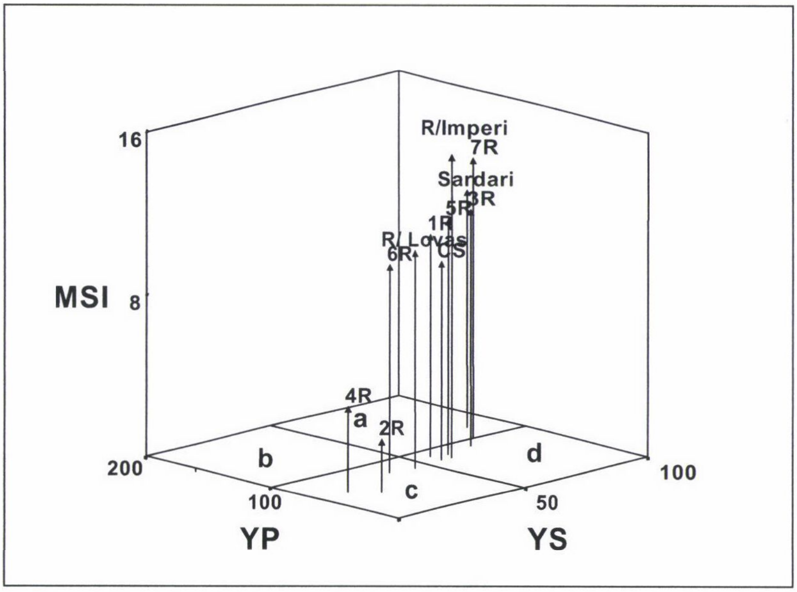

Fig .2. Three-dimensional plot based on Ys, Yp and MSI
(Imperi: Imperial, Lovas: Lovászpatonai, CS: Chinese Spring)

Discussion

Relative water content (RWC) is considered as one of the most useful physiological characters in drought tolerance studies (Dedio, 1975; Morgan, 1989; Singh, 1989; Schonfeld et al., 1988). In the present investigation, although the 6R line exhibited higher RWC, it also had higher RWL. Therefore, in this line the physiological mechanism of water maintenance was poor. On the other hand, lines 7R and 3R, which had high RWC and lower RWL, indicated the presence of gene(s) on chromosome 7R of rye which affected the water balance in the plant (Table 2). Sojka et al. (1981) and Blum (1983) showed that wheat lines with higher water potential also had higher osmotic adjustment potential.

Lines 7R and 3R also had higher stomatal resistance. This trait may allow the plant to maintain higher water status, which may be why the RWC of these two lines was high (Table 2).

The STI criteria was used for screening drought-tolerant genotypes by Fernandez (1992), who reported a positive, significant correlation between the yield under stress and non-stress conditions and the stress tolerance index (STI). The addition lines 7R and 3R produced more yield under both stress and non-stress conditions, so they had higher values of STI.

The quantum yield was high for lines 3R and 5R. Genty et al. (1989) reported a positive correlation between yield and drought resistance and suggested that varieties with higher quantum yield also had higher drought resistance. This is in good agreement with the higher value of quantum yield recorded for 3R and 5R, since these two lines also had high STI (Table 2), reflecting their higher drought tolerance.

Evaluation of the genotypes under stress and non-stress conditions for their germination rate measured by GSI indicated that 7R and 3R also had higher values of GSI. Sapara et al. (1991) showed that lines with higher GSI also had high drought tolerance, so this criterion could be used to select lines for drought tolerance. Bouslama and Schapaugh (1984) used high values of the germination stress index (GSI) for the selection of resistant lines and varieties. Based on STI and GSI, 7R and 3R were selected for drought tolerance.

The multiple selection index (MSI) of lines 7R, 3R and 5R was higher than that of the other lines (Table 2). This reveals that these chromosomes (7R, 3R and 5R) must carry genes, making the plants more drought-tolerant. On the other hand, lines 4R and 2R showed lower MSI, which may show the presence of suppressor gene(s) for the QTLs controlling physiological traits related to drought tolerance. The efficiency of each pair of rye chromosomes in the background of Chinese Spring was calculated as the percentage increase/decrease of their MSI compared to that of CS. This criterion also indicated that chromosomes 7R, 3R and 5R had the highest value of EAC (Table 2).

The check (Sardari), a widely adapted local bread wheat variety from west Iran, is one of the most drought-tolerant varieties of hexaploid wheat. Cluster analysis grouped the addition line 7R, which had high RWC, STI and GSI values, in the same cluster as Sardari (Fig. 1). Three- dimensional representation of the performance of the genotypes (Fig. 2) placed 7R and 3R in a region of the graph with higher yield under both stress and non-stress conditions, and higher STI. The overall ranking of the characters and environments studied in this investigation showed that chromosomes 3R and 7R carry most of the QTLs controlling drought tolerance. It is therefore suggested that these lines be used in breeding programmes to transfer useful traits to a wheat background. Molecular analysis of the progenies could be useful to characterize these lines for the location of the QTLs on each chromosome arm.

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STUDIES ON THE BEHAVIOUR OF SOME FUNGAL DISEASES OF RICE IN THE MANGROVE SWAMP ECOLOGY AT WARRI, SOUTH EASTERN NIGERIA

E. A. MAJI and E. D. IMOLEHIN

NATIONAL CEREALS RESEARCH INSTITUTE, BADEGGI, BIDA, NIGER STATE, NIGERIA

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Studies on the ecological behaviour of *Cochliobolus miyabeanus* (Ito et Kurib.) Drechsl. ex Dast., syn. *Bipolaris oryzae* (Breda de Haan Shoem.), the causal agent of brown spot in rice (*Oryza sativa* L.), were carried out in the tidal mangrove swamp at Warri Experimental Farm, Southeastern Nigeria. A split randomised complete block design with four replications was used. Monthly transplantings from July to September formed the main plot, which was subdivided into control and N-treated subplots. Disease incidence increased when transplanting was delayed. This was probably due to the fact that flowering coincided with environmental conditions favourable for disease development from November to February. Nitrogen fertilization at 40 kg N/ha significantly (P=0.05) reduced *C. miyabeanus* incidence in 1997/1998, but not in the 1998/1999 and 1999/2000 cropping seasons at the same site. The grain yields of ROK 5, a medium-duration improved rice variety (approx. 150 days), were significantly (P=0.05) reduced in late-transplanted crops (September to November) in spite of adequate N fertilization. Mangrove mud was not an important source of *C. miyabeanus* propagules. The incidence of leaf scald caused by *Monographella albescens* (Thum) Parkinson, Sivanesan and Booth syn. *Microdochium oryzae* (Hashioka and Yokogi) Samuels and Hallet, and of leaf smut caused by *Etyloma oryzae* Miyake was generally stimulated by N application.

Key words: mangrove, fungal disease behaviour, rice, incidence

Introduction

In the mangrove swamps around tidal rivers and creeks in Southeastern Nigeria, the cultivation of rice has been practised for a century (Jordan, 1965; Spencer, 1975; Fomba and Singh, 1991). Cultural practices in rice production, which are mainly manual, have not changed significantly with time and the incidence of rice diseases was considered minimal under these low-input subsistence conditions (Deighton, 1996; Raymundo, 1980). However, recent surveys in Nigeria confirmed brown spot disease caused by *Cochliobolus miyabeanus* (Ito et Kurib) Drechl. ex Dastur syn. *Bipolaris oryzae* (Breda de Haan) Shoem was the prevalent fungal disease of rice in tidal and associated grass/sedge mangrove swamps, which are flooded seasonally in the wet season (Fomba, 1996). Mangrove swamps are essentially dry during most of the dry season, especially those adjacent to the tidal mangrove swamps. Brown spot was earlier confirmed as a key disease in the mangrove environment (Fomba, 1987; Fomba and Singh, 1990). The present study was therefore conducted in the 1997/98 to 1999/2000 cropping seasons to define the effect of time of planting, nitrogen application, the salinity and acidity conditions of the field, and some weather parameters on the incidence of *C. miyabeanus* in a mangrove swamp ecosystem at Warri Experimental Farm in Southeastern Nigeria. The incidence of two other common fungal diseases of rice, leaf scald (*Monographella albescence* (Thum) syn. *Microdocium oryzae*) and leaf smut (*Etyloma oryzae*) was also studied.

Materials and methods

A field trial was carried out in the tidal mangrove swamp ecology in Warri, Southeastern Nigeria to study the variation of *C. miyabeanus* occurrence in the 1997/98 to 1999/2000 cropping seasons. The experiment was located in an area which was deep flooded in the catena of the mangroves, mostly with more than 50 cm of standing water at high tide. An improved rice cultivar, ROK 5 (*Oryza sativa* L.), susceptible to brown spot and of medium duration (approx. 150 days), and CK 73 (*O. sativa*), a long duration (approx. 180 days) rice cultivar susceptible to brown spot, were used in the experiment. Seedlings treated with NPK fertilizer were transplanted at the age of 6 weeks into 6 × 2 m plots at a spacing of 20 × 15 cm (33 hills m^{-2}). Monthly transplantings from July to September formed the main plot treatment. These were subdivided into 3 × 2 m control and N-treated subplots in a split-plot design with four replications. Nitrogen was applied to the root zone (20 cm depth) at 0 and 40 kg ha^{-1} as 33% urea solution by injection with a converted Cooper Pegler 3 knapsack pressure sprayer. This reduced the wash-off of applied nitrogen due to tidal interflow (Jones and Dixon, 1979). The non-fertilized subplot treatments simulated the cultivation practices of Nigerian mangrove swamp rice farmers and were separated from the N-fertilized subplots by 0.5 m borders planted to variety CK 73.

To assess the contribution of mangrove swamp soil as a source of inoculum of the pathogen, the number of propagules of *C. miyabeanus* per gram of soil was determined in soil taken from the experimental site and from untreated associated mangrove swamp and upland soils in Warri. The upland soil was taken in the wet season (July, 2000) from the nursery site where the rice seedlings were raised. Soil samples were taken at the corners of a 15 × 15 m quadrat in the dry (April) and wet seasons (July, 2000). A total of 18 samples were taken at each site at 0–5, 5–10, 10–15 and 15–20 cm depth using an auger. For each site the soil samples from each depth were bulked, mixed and kept tightly secured in polythene to avoid drying. The dry season samples were taken in April when the tidal swamps were saline due to salt water incursion from the sea, while the wet season samples were taken in July prior to transplanting, when the swamp contained freshwater from rainfall and floods. The samples were assessed for *C. miyabeanus* propagules. Samples of field and river waters at Warri Experimental Farm were collected in April and July, and sterilized at 1 atmosphere in a portable autoclave. The sterilized water samples were used to assess the conidial germination of *C. miyabeanus* on glass, using sterilized distilled water as a control.

The seasonal variation of *C. miyabeanus* was studied by counting the number of lesions on the top three leaves per rice plant in a hill 2 weeks after flowering and at harvest. Ten such counts were made on 10 randomly selected plants from 10 randomly selected hills per treatment in all replications. The occurrence of leaf scald (*M. albescense*) and leaf smut (*E. oryzae*), the two other prevalent fungal pathogens of rice at the site, was recorded using a 0–9 standardized scale (IRRI, 1996), where 0 indicated no disease and 9 indicated maximum disease incidence. The grain from each net plot was hand-harvested, processed and calculated as kg/ha (adjusted to 14% moisture content). Weather data were also collected during the period of study. The disease incidence and grain yield data were analysed using ANOVA (Snedecor and Cochran, 1967).

A selective medium, developed for the isolation of *Cochliobolus sativus* from wheat soil in Brazil (Reis, 1983), was used to isolate *C. miyabeanus* propagules from the soil samples as follows.

Ten g of soil per sample were suspended in 100 ml of 0.1% agar and 1 ml aliquots of a 10^{-1} dilution were pipetted onto 20 ml of the congealed selective medium containing one-quarter strength potato sucrose agar, fungicides (benomyl, dicloran and captan) and antibiotics (Streptomycin and Neomycin) in 9 cm Petri dishes. To prevent contamination of the samples by other microorganisms the dishes were gently shaken and tilted to spread the soil suspension uniformly over the agar surface. The treatments, comprising soil samples from the four depths, 0–5, 5–10, 10–15 and 15–20 cm, were laid out in a random complete block design with four replications. The treated dishes were incubated under 20 W white fluorescent bulbs on a 6-hour cycle per day to stimulate sporulation (Leach, 1961; Klomp, 1977). The Petri dishes were examined every 2 days for fungal growth, and colonies of *C. miyabeanus* (dark brown or olivaceous) were identified and counted. Smears from 10-day-old cultures were made aseptically on flame-sterilized glass slides and stained with 5% cotton blue lactophenol for examination under a compound microscope to assess the number of *C. miyabeanus* colonies per gram of soil.

To determine the effect of mangrove swamp field conditions (salinity and acidity) on the conidial germination of *C. miyabeanus,* 2-week-old mature conidia of the pathogen cultured on glucose peptone agar (Fomba, 1987) were harvested into sterile distilled water and the concentration was determined with a haemocytometer. In the various treatments field- and river-collected water, sterilized water samples and sterile distilled water as a control were added to the conidial suspension in 15 mm diameter vaseline rings with an oxford micro-pipette. A total volume of 50 µl was used per treatment per vaseline ring with a concentration of conidia of 80×10^3 ml. Each treatment suspension was replicated twice. The treated slides were incubated under white fluorescent light for 24 hours and then fixed on a hot plate.

The conidial smear was stained with 5% cotton blue-lactophenol for microscopic assessment. Condidia were enumerated for percentage germination and germ tube length measured with an American Optical model 426 micrometer eyepiece. Between 20 and 35 conidia were counted per microscopic field when the individual fields did not have sufficient conidia. Altogether 10 such fields and/or transects were assessed per treatment in each replicate, and 50 germ tube lengths were measured. A conidium was considered to have germinated when the germ tube equalled or surpassed the length of the conidium. Conidial germination and germ tube length data were analysed using confidence limits and Student's t-test, respectively (Blakeman, 1997).

Results

The severity of *C. miyabeanus* infection on the rice plants varied considerably from season to season and with the time of planting (Table 1). The high disease score registered for the September-transplanted rice crop in 1997/98 corresponded with relatively high monthly temperature minima, light but frequent rain and high relative humidity at night in December 1997, when the crop flowered (Table 2). In the 1998/99 season the maximum disease score was recorded in the November-transplanted rice crop which flowered in February 1999. In the 1999/2000 cropping season, maximum disease incidence was again noted in the September-transplanted rice crop although the disease pressure was less (Table 1). Each of these disease maxima were correlated with similar environmental conditions with relatively high temperature minima, high relative humidities at night and drizzly weather (Table 2). Nitrogen fertilization at 40 kg N/ha only significantly (P=0.05) reduced the *C. miyabeanus* severity in the 1997/98 cropping season; this relationship was not observed in the 1998/99 and 1999/2000 cropping seasons at the same site.

Table 1
Seasonal variation in brown spot severity and grain yield of rice cultivar ROK 5 (*O. sativa*) in a tidal mangrove swamp at Warri, Southeastern Nigeria

Transplanting /Flowering date	1997/98				1998/99				1999/2000			
	Grain yield (kg/ha)*		Disease index**		Grain yield (kg/ha)*		Disease index**		Grain yield (kg/ha)*		Disease index**	
	UF	F	UF	F	UF	F	UF	F	UF	F	UF	F
July/Oct.	2480	2660	47	41	2660	3460	30	32	2530	3890	10	10
Aug./Nov.	2230	2990	32	29	2130	2390	39	43	2500	3670	14	17
Sept./Dec.	1390	2010	31	57	1940	2030	20	22	1670	2120	33	34
Oct./Jan.	1490	1700	44	37	1070	907	44	40	1000	1480	29	32
Nov./Feb.	1680	1820	63	44	387	347	64	67	1290	1510	29	27
$LSD_{5\%}{}^{+}$	355		11		508		11		516		8	
$LSD_{5\%}{}^{++}$	527		7		7.5		7.5		614		7.5	

* = Adjusted to 14% moisture content. ** = Lesion count per leaf per plant; mean of 4 replications. UF = Unfertilized; F = Fertilized with 40 kg N/ha. $^{+}$for comparison of date of planting; $^{++}$for comparing effect of nitrogen at each planting date. Mean values of combined analysis for the years 1997/1998–1999/2000.

Table 2
Seasonal variation and effect of rainfall on brown spot severity on rice cultivar ROK 5 (*O. sativa*) in a tidal mangrove swamp at Warri, southeastern Nigeria

Transplanting /Flowering date	1997/98				1998/99				1999/2000			
	RH %	Rainfall (mm)	Min. t. (°C)	Disease index	RH %	Rainfall (mm)	Min. t. (°C)	Disease index	RH %	Rainfall (mm)	Min. t. (°C)	Disease index
July/Oct.	90a	195a	22b	45c	90a	290a	21a	30c	90a	195a	21a	10c
Aug./Nov.	92a	30c	22a	50b	90a	170b	21a	40b	90a	100b	21a	15c
Sept./Dec.	91a	60b	20b	65a	80c	10d	19c	20d	71b	10c	19b	30a
Oct./Jan.	82b	10d	19c	38d	88b	10d	20b	38b	78b	10c	17b	28a
Nov./Feb.	82b	10d	19c	30e	88b	30c	20b	60a	85a	10c	21a	25a

Figures with a similar letter in each column are not significantly different according to Duncan's Multiple Range Test (P=0.05). *Number of lesion counts/leaf/plant.

Monthly grain yields were decreased by delayed transplanting especially from September to November. Nitrogen-treated plots out-yielded the control plots (P=0.05) except in the October and November-transplanted rice crops in 1998/99 (Table 1).

Leaf scald (*Monographella albescens*) and leaf smut (*E. oryzae*) also showed seasonality in incidence (Table 3). Both diseases decreased in severity with delayed transplanting. Nitrogen fertilization significantly (P=0.05) stimulated leaf scald development throughout the study period, but the effects on leaf smut were rather inconsistent. Leaf scald appeared to be particularly sensitive to the onset of dry season conditions, but leaf smut was much less affected (Table 2).

Propagules of *C. miyabeanus* were recovered from an associated mangrove swamp at Warri Experimental Farm in the order of 25 and 194 colony-forming units (c.f.u.) per gram of soil in the dry and wet seasons, respectively. However, 1810 c.f.u per gram of soil were obtained from an adjacent nursery site on the upland at Warri in the 1999 wet season.

The depth of soil sampling did not significantly (P=0.05) affect the amounts of *C. miyabeanus* propagules recovered from an associated mangrove swamp, although more propagules (475 c.f.u. per gram of soil) were obtained from 0–5 cm depth compared to the remaining samples at 5–10, 10–15 and 15–20 cm depths, from which 100 c.f.u. per gram soil were obtained in the wet season. In contrast, 4880, 1688, 325 and 375 c.f.u. per gram of upland soil were recovered at Warri at the four depths sampled.

The *in vitro* conidial germination of *C. miyabeanus* in sterilized field and river water samples collected in the dry season was significantly (P=0.05) lower than in the control, but this was not the case in the wet season sample (Table 4). However, germ tube growth was not significantly affected.

Table 3
Incidence of *Monographella albescens* and *Etyloma oryzae* on rice (Cv. ROK 5) in a tidal mangrove swamp at Warri, Southeastern Nigeria

Transplanting /Flowering date	1997/98				1998/99				1999/2000			
	Monographella albescens		*Etyloma oryzae*		*Monographella albescens*		*Etyloma oryzae*		*Monographella albescens*		*Etyloma oryzae*	
	UF	F	UF	F	UF	F	UF	F	UF	F	UF	F
July/Oct.	1.4	6.0	6.8	6.8	1.0	6.2	7.2	7.5	1.2	6.0	5.0	8.0
Aug./Nov.	1.4	5.7	6.4	6.9	1.6	4.2	5.5	6.0	0	2.5	4.2	5.5
Sept./Dec.	0.2	6.0	6.7	7.1	1.5	3.1	7.5	7.5	0	0	7.2	6.8
Oct./Jan.	0.2	5.1	5.4	6.2	0	0	3.0	7.8	0	0	1.6	3.2
Nov./Feb.	0	0	6.2	5.6	0	0	0.4	0.8	0	0	1.5	2.5
$LSD_{5\%}{}^{+}$	0.6		0.7		2.6		1.8		1.1		1.4	
$LSD_{5\%}{}^{++}$	0.7		7.5		1.1		1.4		2.0		1.1	

$^{+}$ for comparison of date of planting; $^{++}$for comparing effect of nitrogen at each planting date. Disease evaluated on the basis of the IRRI standard evaluation system for rice; mean of 4 replications. UF = Unfertilized, F = Fertilized with 40 kg N/ha.

Table 4
Effect of dry and wet season field- and river-collected water samples at Warri experimental farm on the *in vitro* conidial germination of *Cochliobolus miyabeanus*

Treatment	Percentage germination with confidence limits*
Control (sterile distilled water)	56.8 (53.5–60.2)
Field water - dry season April (sterile)	37.6 (36.0–39.2)
Field water - wet season, July (sterile)	53.6 (53.0–54.8)
River water - dry season April (sterile)	37.8 (36.2–39.4)
River water - wet season July (sterile)	53.8 (52.5–55.0)

*Based on the mean of 10 microscopic fields and/or transects per treatment, replicated twice (confidence limits, P=0.05); percentage conidial germination arcsin transformed.

Discussion

The seasonal variations observed in *C. miyabeanus* disease severity could be explained in several ways. Firstly, the increased severity of the disease in the late-transplanted rice crops in a tidal mangrove swamp at Warri was probably due to increased stresses due to salinity and acidity during the dry season. Salinity has been shown to pre-dispose the rice crop to attack by *C. miyabeanus* (Mueller, 1974). The pathogen is a weak parasite which attacks stressed rice plants (Ou, 1985; Fomba and Singh, 1991). Secondly, the generally favourable weather conditions, consisting of high average monthly temperature minima, long and abundant leaf wetness periods and relatively high relative humidities at night from November to February at Warri may have promoted rapid disease development in late-maturing crops of late-transplanted cv. ROK 5. Thirdly, additional pathogen inoculum originating from rice stubble and dispersed by the wind from sources other than the experimental site contributed to the disease build-up in late-transplanted rice crops, i.e. September to November.

The absence of significant differences in grain yield between the N-treated and control plots in the last two monthly transplantings in 1998/99 contradicts the results of the 1997/98 and 1999/2000 cropping seasons. Nitrogen fertilization has been noted to increase rice grain yields in mangrove swamps in southeastern Nigeria and at several places in the West African subregion (WARDA, 1983). However, grain reductions were almost always observed when rice crops were transplanted late, especially between September to November at Warri (Crawford, 1964), probably due to increasing brown spot severity and soil stresses. This situation is usually aggravated by reduced rainfall and the early incursion of saline water onto the swamps at Warri, as was observed in 1998/99.

The seasonal variations in leaf scald and leaf smut severity in the tidal mangrove swamp at Warri were also strongly influenced by the prevailing meteorological and cultural factors. The effect of the local harmattan winds (north east trade winds) in January to February, resulting in decreased relative humidity, may have adversely affected the development of both diseases during this period, especially that of leaf scald, which is favoured by high relative humidities (Nottechem and Baudin, 1981; Ou, 1985; Fomba and Singh, 1991).

Mangrove mud was not an important source of *C. miyabeanus* propagules except at the Warri associated mangrove swamp where the pathogen was recovered. However, the pathogenicity of the isolates was not tested. Swart (1988) in Mozambique, and Fomba and Singh (1991) in Sierra Leone also recovered *C. miyabeanus* propagules from similar associated mangrove swamp soils. Since *C. miyabeanus* is an aerobic pathogen (Kulkarni et al., 1980), the dry season conditions in these swamps would favour the survival of secondary inoculum from rice stubble in the swamps and surrounding uplands. *Cochliobolus miyabeanus* was observed infecting rice nodes and adjacent senescing tissues in both tidal and associated mangrove swamps in southeastern

Nigeria. The absence of *C. miyabeanus* propagules in tidal mangrove mud at Warri may have been due partly to the generally anaerobic conditions and soil toxicity problems in these swamps caused by the tidal interflow throughout the year and salinity/acidity in the dry season. Swart (1988) also failed to recover *C. miyabeanus* propagules in tidal mangrove swamps in Mozambique. Fomba (1987) observed that soil salinity and acidity may adversely affect the occurrence of fungal plant pathogen propagules, including *C. miyabeanus,* in tidal swamps.

The presence of hydrogen sulphide in the tidal swamp soil was thought to reduce fungal propagules in Mozambique (Swart, 1988). A similar phenomenon would probably occur in tidal mangrove swamps in Warri and elsewhere in the WARDA region.

The pathogen is also subject to microbial antagonism in rice soils (Rangaswami and Ramalingam, 1962). Similar antagonism may occur in swamps in southeastern Nigeria.

In conclusion, tidal mangrove mud was not an important source of *C. miyabeanus* propagules compared to either associated mangrove swamps or upland soils. The timely transplanting of rice (from July to August), using a medium-duration rice cultivar such as ROK 5 (*O. sativa*) and adequate levels of applied N, will enhance good rice growth and minimize *C. miyabeanus* attack. However, early-transplanted rice crops appear particularly susceptible to both leaf scald and leaf smut, especially under N fertilization. The economic importance of leaf scald and leaf smut diseases is yet to be defined under mangrove swamp conditions in Nigeria.

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BROADLEAF WEED CONTROL IN CHICKPEAS (*CICER ARIETINUM*), FABA BEANS (*VICIA FABA*) AND LENTILS (*LENS CULINARIS*)

H. Z. GHOSHEH and M. K. EL-SHATNAWI

FACULTY OF AGRICULTURE, JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, JORDAN

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Field experiments were conducted to evaluate the efficacy of herbicides applied alone or in combination for broadleaf weed control in chickpeas, faba beans and lentils. Herbicide applications of metribuzin pre-emergence; pendimethalin pre-emergence; bentazon post-emergence; metribuzin pre-emergence followed by bentazon post-emergence; and pendimethalin pre-emergence followed by bentazon post-emergence were examined and compared to weedy and hand-weeded plots. In chickpeas, metribuzin provided substantial control of broadleaf weeds; however, some injury was observed. Pre-emergence applications in faba beans provided substantial control of broadleaf weeds, and the bean yield was comparable to hand-weeded plots. No additional advantage was observed from combining bentazon with pre-emergence applications. Lentil plants were sensitive to herbicide applications, which caused crop injury and reduced the seed yield significantly.

Key words: chickpeas, faba beans, lentils, weeds, weed control, herbicide, broadleaf weeds

Introduction

Chickpeas (*Cicer arietinum* L.), faba beans (*Vicia faba* L.) and lentils (*Lens culinaris* Medik.) are the major legume crops in the developing countries of Asia and Africa (Duc, 1997; Nygaard and Hawtin, 1981; Williams and Singh, 1987). Yields of all three crops are low when compared to values reported in developed countries. For example, the average seed yield for faba beans in Jordan was 0.93 t/ha during the period 1990–1995 (Anonymous, 1995), which is rather low and requires improvement. Likewise, yields of chickpeas and lentils are among the lowest in the world (Haddad, 1984; Haddad and Arabiat, 1985). These low yields are attributed to factors of low and erratic rainfall, planting of low-yielding cultivars and poor cultural practices. Weed control, in particular, is ignored by many legume farmers, although the devastating effects are encountered every year. Weeds that compete with the crop for light, nutrients and moisture will definitely reduce yields (Radosevich et al., 1997).

Lentils and faba beans are normally planted in winter in tropical and subtropical areas. Chickpeas, on the other hand, were until recently planted in early spring as a summer-season crop. Producers tend to delay thee sowing of both lentils and chickpeas to avoid the heavy weed infestations that accompany winter sowing (Haddad, 1984; Haddad and Arabiat, 1985). Jordanian farmers are hesitant to use herbicides due to their uncertainty of the achieved weed control levels, possible crop injury, lack of experience in applying herbicides at the proper time and rate, and also due to the higher costs associated with using herbicides.

The production of legume crops in general is impaired by harvesting difficulties and inadequate weed control. Hand weeding is expensive, even in developing countries, where labour costs are minimal. Thus, chemical control is favoured by weed management specialists. However, until now only few chemical products have been registered for weed control in chickpeas, faba beans or lentils (Vencill, 2002). Nevertheless, several herbicides not registered for these crops have been tested and are used in certain countries. Caballero et al. (1992) observed acceptable levels of weed control in faba beans when alachlor plus linuron, EPTC, trifluralin, dimethalin and dinoseb, simazine, pendimethalin and terbutylazine herbicides were tested in Spain. In Jordan, sethoxydim and fluazifop-butyl were found effective for grass weed control in chickpeas. The weed control spectrum of these two herbicides was broadened to some extent by combining terbutryn at a rather high rate (Yasin et al., 1995). Otherwise, few reports described the weed control efficacy of herbicide applications in legume crops elsewhere in the world (Butler and Alexander, 1987; Hassan, 1987; Friesen and Wall, 1986; Vaishya et al., 1999). Therefore, the objective in this research was to evaluate the efficacy of several commercially available herbicides for broadleaf weed control in chickpeas, faba beans and lentils grown in northern Jordan.

Materials and methods

Field experiments were conducted at the Jordan University of Science and Technology campus (JUST) and the Maru Experimental Research Station (Maru) in northern Jordan. The experiments were conducted on alkaline soils that have low organic matter content. The first site (JUST) was a semi-arid location with an average annual precipitation of 230 mm. The second site (Maru) was more humid with a long-term average annual precipitation of 310 mm. In order to closely simulate the farm situation, faba beans were planted at JUST under irrigation in the 1998/99 and 1999/2000 seasons, whereas chickpeas and lentils were grown in two separate experiments at Maru in the 1998/99 season under rainfed conditions. All the experiments were arranged in randomized complete block designs with three replicates. Plots of 2.5 by 4 m separated by a 1 m buffer area were planted with faba beans cv. Major on Nov. 26, 1997 and Dec. 10, 1998. The seeds were planted 6 cm deep with 20 by 20 cm spacing on both sides of irrigation pipes laid at 1 m intervals. Similar plots were utilized in the chickpea and lentil experiments. Both crops were planted at seeding rates of 100 kg/ha. Chickpeas (cv. Jubeiha 3) and lentils (cv. Jordan 1) were planted 4 cm deep in 35 rows at Maru on Dec. 2nd, 1997. Irrigation, insect control and fertilizer applications were performed as recommended. All the experiments were treated 4 weeks after crop emergence with fluazifop-butyl at 500 g ai/ha to control grass weeds. The experiments were terminated in mid-May of each season.

The treatments evaluated were weedy check (untreated), hand-weeded (6 times during the growing season at two-week intervals), metribuzin pre-emergence (PRE) (750 g ai/ha), pendimethalin (PRE) (750 g ai/ha), bentazon post-emergence (POST) (750 g ai/ha), a combination of metribuzin PRE (750 g ai/ha) followed by bentazon POST (750 g ai/ha), and a combination of pendimethalin PRE (750 g ai/ha) followed by bentazon POST (750 g ai/ha). These rates were selected to coincide with low to medium herbicide rates recommended for other similar large-seeded legumes (Ashton and Monaco, 1991). Metribuzin is a selective asymmetrical triazine herbicide that is used to control annual broadleaf weeds in several crops. Pendimethalin is a dinitroaniline herbicide that controls certain broadleaf weeds and does not require soil incorporation. Bentazon is a selective post-emergence herbicide used to control a number of annual and perennial broadleaf weeds in many large-seeded legume crops (Ashton and Monaco, 1991).

In all the experiments, the pre-emergence treatments were applied one day after planting and before irrigating the plots, or before rain occurred. The post-emergence treatments were applied 25 days after crop emergence when plant heights were 5–8 cm for crops and 3–5 cm for weeds. All the herbicides were applied using a pressurized CO_2 backpack sprayer at a spray volume of 187 l/ha. The broadleaf weed infestations at JUST were natural stands of *Amaranthus* spp., *Cardaria draba* (L.) Desv., *Diplotaxis erucoides* (L.) DC. and *Trigonella arabica* Del. The broadleaf weeds present at Maru were mainly *Malva parviflora* L., *Sinapis arvensis* L., *Vaccaria pyramidata* Medik. and *Tetragonolobus palestinus* Boiss.

The measurements recorded each year included visual weed control ratings recorded 3 and 6 weeks after the POST treatments on a scale from 0 (no weed control) to 100 % (complete weed control). Crop injury was recorded 3 and 6 weeks after the POST treatments on a scale from 0 (no injury) to 100 % (dead plants) in the chickpea and lentil experiments. Plant height was measured as the vertical distance from the ground surface to the uppermost leaf tip at 3 and 6 weeks after the POST treatments. Before harvest, weed fresh biomass was determined in random 1-m^2 quadrates per plot. Fresh biomass was determined for faba beans by hand-harvesting whole plants at the soil surface in 1-m^2 quadrates from each plot. Quadrates of similar size were used to determine the dry aboveground biomass of chickpeas and lentils. Seed weights for chickpeas, faba beans and lentils were also determined.

Analysis of variance procedures were performed on all the data. The data for the two years were combined and the averages of the two years are presented. The weedy check plots were omitted from the analysis of the visual weed control ratings, and the hand-weeded plots from that of weed biomass. Means were separated according to Fisher's LSD test at $P \leq 0.05$.

Results and discussion

Chickpeas

Crop injury was observed when herbicides were used for weed control in chickpeas. The greatest injury was observed when metribuzin PRE was followed by bentazon POST (Table 1). Metribuzin alone caused significant crop injury, but this injury was of lower magnitude later in the season. Injury symptoms appeared as bronze casting and chlorosis or necrosis. Visual weed control ratings recorded 3 or 6 weeks after post-emergence applications indicated that metribuzin alone or in combination had excellent broadleaf weed control (Table 1). Pendimethalin alone or in combination did not provide extended periods of weed control. These results disagree with those reported by Thakar et al. (2000) and Vaishya et al. (1999) who observed excellent weed control after pendimethalin applications. Rainfall in Maru is unevenly distributed, which could contribute to the lower efficacy of pendimethalin in the present experiment. Bentazon, on the other hand, provided poor weed control when evaluated 3 or 6 weeks after application. Weed suppression, as indicated by weed fresh weight, also indicated that the best control was acheived in the hand-weeded plots followed by metribuzin and metribuzin+bentazon.

Table 1
Influence of herbicide treatments on crop injury (%), weed control ratings (%) and weed fresh weights (g/m^{-2}) in chickpeas

Treatment and application timing	Rate (g ai/ha)	Crop injury (1)	Crop injury (2)	Weed control (1)	Weed control (2)	Weed fresh weight
Weedy check	–	0 c*	0 c	–	–	117a
Hand-weeded (biweekly)	-	0 c	0 c	92 a	90 a	-
Metribuzin (Pre)	750	17 b	10 b	90 a	90 a	9 c
Pendimethalin (Pre)	750	7 c	6 bc	75 b	55 bc	40 b
Bentazon (Post)	750	6 c	2 c	40 c	45 c	82 a
M.buzin (Pre) + bent. (Post)	750 + 750	52 a	42 a	90 a	95 a	7 c
Pend. (Pre) + bent. (Post)	750 + 750	15 b	2 c	82 b	65 b	24 bc

Abbreviations: Pre = Pre-emergence; Post = Post-emergence; (1) 3 weeks after post-emergence treatment (2) 6 weeks after post-emergence treatment. *Means followed by similar letters within a column do not differ according to Fisher's LSD test ($P \leq 0.05$).

Chickpea plant heights recorded 3 weeks after the post-emergence treatments indicated that metribuzin PRE followed by bentazon POST caused stunting (Table 2). These plants, which also exhibited symptoms of bronze casting and necrosis, were not able to recover. The total dry and seed weights of such plants were comparable to those in the weedy check plots that were exposed to higher levels of weed infestation. Plots treated with metribuzin alone had total dry weight and seed yield values that were comparable to the weed-free plots.

In terms of weed management and crop yields, the results of this experiment indicate that hand weeding can be substituted by pre-emergence applications of metribuzin at 750 g ai/ha. However, the injury reported in chickpeas after this treatment is a matter for concern. Chickpeas grown in rainfed areas may encounter unpredictable, extended periods of either drought or wet weather. Under such circumstances, the observed injury may be magnified.

Faba beans

Visual weed control ratings recorded 3 or 6 weeks after post-emergence application revealed that pre-emergence herbicides had very good broadleaf weed control (above 80%) (Table 3). Pre-emergence herbicides provided extended periods of weed control that exceeded 65 days from the beginning of the growing season. The weed fresh weight measured at the end of the season supported this fact (Table 3). These results suggest that the end of the critical period for controlling the prevailing broadleaf weeds in faba beans is around 65 days after crop emergence under the given experimental conditions. The critical period of weed control is the period during which weeds should be controlled to prevent yield losses. Therefore, weed control at other times may not be necessary (Radosevich et al., 1997). Post-emergence applications of bentazon did not provide adequate control (49% or 66% at 3 and 6 weeks after POST application, respectively) (Table 3). The application of bentazon POST after either metribuzin PRE or pendimethalin PRE did not improve weed control. However, a dramatic reduction in weed fresh weight was observed when metribuzin treatment was followed by bentazon application.

Table 2
Influence of herbicide treatments on chickpea height (cm), total dry weight (g/m^{-2}) and seed yield (g/m^{-2})

Treatment and application timing	Rate (g ai/ha)	Plant height (1)	Total dry weight	Seed dry weight
Weedy check	–	39 a*	63 c	20 c
Hand-weeded (biweekly)	–	39 a	140 a	90 a
Metribuzin (Pre)	750	38 a	136 a	91 a
Pendimethalin (Pre)	750	30 a	105 b	68 b
Bentazon (Post)	750	37 a	85 bc	64 b
M.buzin (Pre) + bent. (Post)	750 + 750	23 b	75 c	36 c
Pend. (Pre) + bent. (Post)	750 + 750	35 a	108 b	72 b

For notes see Table 1

Table 3
Influence of herbicide treatments on weed control visual ratings (%) and weed fresh weight (g/m^{-2}) in faba beans

Treatment and application timing	Rate (g ai/ha)	Weed control (1)	Weed control (2)	Weed fresh weight
Weedy check	–	–	–	924 a
Hand-weeded (biweekly)	–	95 a*	94 a	–
Metribuzin (Pre)	750	89 a	80 b	201 cd
Pendimethalin (Pre)	750	85 a	85 b	308 c
Bentazon (Post)	750	49 b	66 c	536 b
M.buzin (Pre) + bent. (Post)	750 + 750	92 a	80 b	94 d
Pend. (Pre) + bent. (Post)	750 + 750	90 a	90 a	253 c

For notes see Table 1

The plant height data recorded 3 weeks after the post-emergence treatments showed that bentazon applied alone or in combination caused stunting (Table 4). Hovewer, these plants, which also exhibited symptoms of bronze casting and chlorosis, were able to recover, as the reductions were insignificant when the heights were recorded 6 weeks after the post-emergence applications (data not presented), though the reduction in biomass values indicates that this recovery was not complete (Table 4). On the other hand, plants treated pre-emergence with the herbicides metribuzin or pendimethalin had dry yields equal to those with hand-weeded plots (Table 4). Hassan (1987) also reported yield advantages when metribuzin was used in faba beans. Caballero et al. (1992) reported increases in the faba bean yield over the weedy control following pendimethalin use. The broadleaf weed suppression in bentazon-treated plots was not sufficient to eliminate yield losses, since the yield was much lower than that harvested from weed-free plots. Adding bentazon to pre-emergence herbicides improved weed suppression, but stunting injury reduced yields. The results of this experiment indicate that hand-weeding could be substituted by the application of metribuzin or pendimethalin under conditions similar to those prevailing in the present experiments.

Table 4
Influence of herbicide treatments on faba bean height (cm), fresh weight (g/m^{-2}) and dry yield (g/m^{-2})

Treatment and application timing	Rate (g ai/ha)	Plant height (1)	Total dry weight	Seed dry weight
Weedy check	–	35 d*	2500 c	295 d
Hand-weeded (biweekly)	–	45 a	5954 a	434 a
Metribuzin (Pre)	750	43 b	5365 ab	420 a
Pendimethalin (Pre)	750	46 a	5832 a	415 a
Bentazon (Post)	750	39 c	4571 b	347 c
M.buzin (Pre) + bent. (Post)	750 + 750	32 de	4596 b	380 b
Pend. (Pre) + bent. (Post)	750 + 750	29 e	4711 b	395 b

For notes see Table 1

Lentils

Crop injury was observed in lentils following herbicide applications, the symptoms (stunting, chlorosis and bronze blasting being magnified as the season progressed (Table 5). The sensitivity of lentils to herbicides confirmed previous reports (Butler and Alexander, 1987). On the other hand, plots subjected to metribuzin PRE alone or followed by bentazon POST were characterized by very good control early in the season, but this control was not extended throughout the growing season when new flushes of weeds were observed. Control levels were less than adequate when ratings were performed 6 weeks after post-emergence applications (Table 5). Although the weed biomass values for all the treatments were lower than in the weedy checks, visual control ratings revealed that none of the treatments provided season-long control. Lentil plants, that have small stature, are apparently not able to compete effectively with the prevailing broadleaf weeds.

The lentil plant heights recorded 3 weeks after the post-emergence treatments showed that all the herbicide applications caused stunting (Table 6). These plants, which also exhibited symptoms of bronze casting and necrosis, were not able to recover, as shown by the heights recorded 6 weeks after the post-emergence treatments (data not shown). In this experiment, lentil plants subjected to herbicide applications had lower dry weight or seed dry weight when compared to the weed-free plots (Table 6). The injury from herbicide applications and the inadequate weed control levels were obviously devastating to the crop. These results are in agreement with Yasin et al. (1995), who reported toxic effects from metribuzin applications. Lowering the metribuzin rates, as done by Friesen and Wall (1986), may avoid crop injury, but the present results suggest that weed control will not be adequate. Therefore, none of the tested herbicides can be recommended for use in lentil production under similar circumstances.

Table 5
Influence of herbicide treatments on crop injury (%), weed control ratings (%) and weed fresh weights (g/m^{-2}) in lentils

Treatment and application timing	Rate (g ai/ha)	Crop injury (1)	Crop injury (2)	Weed control (1)	Weed control (2)	Weed fresh weight
Weedy check	–	0 c*	0 c	–	–	67 a
Hand-weeded (biweekly)	–	0 c	0 c	92 a	92 a	–
Metribuzin (Pre)	750	25 a	35 ab	87 a	65 b	15 b
Pendimethalin (Pre)	750	15 a	32 ab	67 b	46c	31 b
Bentazon (Post)	750	15 b	27 b	52 c	42 c	41 b
M.buzin (Pre) + bent. (Post)	750 + 750	27 a	50 a	85 a	65 b	17 b
Pend. (Pre) + bent. (Post)	750 + 750	22 a	45 a	68 b	55 bc	24 b

For notes see Table 1

Table 6
Influence of herbicide treatments on plant height (cm), total dry weight (g/m^{-2}) and seed yield (g/m^{-2}) in lentils

Treatment and application timing	Rate (g ai/ha)	Plant height (1)	Total dry weight	Seed dry weight
Weedy check	–	32 a*	154 c	105 c
Hand-weeded (biweekly)	–	34 a	243 a	172 a
Metribuzin (Pre)	750	23 c	195 b	145 b
Pendimethalin (Pre)	750	30 b	210 b	135 b
Bentazon (Post)	750	29 b	217 b	116 c
Metribuzin (Pre) + bentazon (Post)	750 + 750	22 c	190 bc	140 b
Pendimethalin (Pre) + bentazon (Post)	750 + 750	28 b	200 b	149 b

For notes see Table 1

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GROWTH AND YIELD MAINTENANCE IN BREAD WHEAT BY SEED PRIMING UNDER LATE-SOWN CONDITIONS

S. KANT, P. VERMA and S. S. PAHUJA

DEPARTMENT OF AGRONOMY, CCS HARYANA AGRICULTURAL UNIVERSITY, HISAR, INDIA

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In the sub-tropical regions of India, the 1st to 3rd week of November is the optimum time for sowing wheat. A delay in sowing due to various factors causes a substantial yield reduction. Seeds of four wheat varieties (Sonak, UP 2338, Raj 3765 and PBW 343) were subjected to seed priming treatments involving water, salts, growth regulator and the sowing of sprouted seed under late-sown conditions during the winter seasons of 1998–99 and 1999–2000. The sowing of sprouted seeds resulted in significantly more rapid emergence of seedlings, accompanied by higher grain and straw yields. Seeds primed with IAA, KCl, water, $ZnSO_4$ and Na_2SO_4 followed in this order. The lowest seedling emergence and grain yield were obtained for unprimed seeds. Seedling emergence was higher in the variety Sonak, while Raj 3765 and UP 2338 had higher leaf water, osmotic and turgor potentials during the 1998–99 season. The variety PBW 343 produced significantly higher grain and straw yields in the 1999–2000 season.

Key words: seed priming, wheat varieties, late sowing, growth rate, yield

Introduction

The optimum sowing time for wheat (*Triticum aestivum* L.) in the major wheat growing areas in India (North-Western and Central zones) is the first three weeks of November. A yield reduction of 0.7% per day occurs in these regions when sowing is delayed beyond this time (Ortizmonasterio et al., 1994). However, wheat sowing is generally delayed till the end of December due to the prevailing long duration multiple cropping patterns and the untimely availability of inputs (Kahlon et al., 1992; Karim et al., 1999; Singh and Uttam, 1994). Late-sown wheat encounters low temperature at sowing time that may delay the emergence of seedlings and the crop gets a shorter growing period due to forced maturity due to high temperature in the month of April (Bhati and Rathore, 1986; Kahlon et al., 1992). The wheat plant develops a less extensive root system under late-sown conditions and may be more vulnerable to drought and heat (Ehdaie and Waines, 2001). The sub-optimal plant population, poor vegetative growth and shorter grain development period under such conditions result in lower grain yield.

The growing of suitable short duration varieties is a promising way of maintaining yields in wheat under delayed sowing (Nainwal and Singh, 2000). In addition, the quicker, better establishment of seedlings may enhance vegetative growth and crop development. This might be possible by seed priming, i.e. by soaking the seed, followed by surface drying and sowing next

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day (Musa et al., 2001). Seed priming helps to ensure rapid emergence (Kahlon et al., 1992), enhanced seedling establishment, optimum crop stand and better tillering (Harris et al., 2001). The objective of testing different seed priming treatments was to maintain the growth and yield of wheat at a level comparable to timely sown conditions. The sowing of water-soaked, sprouted seeds avoids a delay in emergence (Bhati and Rathore, 1986). Priming with various salts (KCl, $ZnSO_4$ and Na_2SO_4) or with a growth regulator was done to test their additional beneficial roles in maintaining osmotic and turgor potential.

Materials and methods

Experimental design and growth conditions

Two field experiments were conducted at the research farm of CCS Haryana Agricultural University, Hisar (India), located at 29°10' N latitude and 75°46' E longitude, at an altitude of 215.2 m. The experimental design was a split plot (three replicates), with the varieties in the main plots and seed primings as sub-plot treatments. Four wheat varieties (Sonak, UP 2338, Raj 3765 and PBW 343) of different growth habit and maturity duration were grown. Sonak and Raj 3765 are recommended for late-sown conditions and UP 2338 for medium to late sowing, while PBW 343 is best suited for timely sown conditions in the region where the experiment was conducted. The seeds of all four varieties were obtained from the plant breeding department of the university, and were produced in the same season and under similar cultivation conditions. The seed priming treatments included (i) water soaking, (ii) sprouted/pre-germinated seeds, (iii) potassium chloride (KCl) 2% solution, (iv) zinc sulphate ($ZnSO_4$) 2% solution, (v) sodium sulphate (Na_2SO_4) 2% solution, (vi) indole-3-acetic acid (IAA) 200 ppm solution, (vii) dry seed sowing (control). The chemicals for seed priming, their concentrations and soaking duration were selected based on a review of previous research papers and preliminary short duration experiments conducted under controlled laboratory conditions. For the priming treatments the seeds were soaked for 18 hours at room temperature (12–20°C) by putting 1 kg dry seeds in 1 l water or solution. The excessive solution was drained off and the seeds were washed with tap water to remove salt adhering on the seed surface. The seeds were then put in the shade for six hours to dry off surplus water, so that the seeds would not stick together at the time of sowing. Sprouting was done by soaking the seeds in an equal volume of water for 12 hours, then removing excess water and enclosing the seeds in moist jute bags. The water was sprinkled on the jute bags regularly at six- hour intervals till the initiation of sprouting. The sowing was done with a conventional plough in rows spaced 20 cm apart, during the third week of December, i.e. during the winter seasons of 1998–99 and 1999–2000. In each plot the seed was applied at the rate of 100 kg dry seed ha^{-1}. The soil of the experimental field was sandy loam and slightly alkaline in reaction. The standard recommended tillage management, fertilizer and irrigation application, plant protection measures and other cultural practices were carried out during the crop growing season.

Observations

The seedling emergence was counted from one-metre row lengths at three randomly selected places in each plot. Relative growth rate (RGR) was calculated using the following formula:

$$\text{RGR (mg g}^{-1}\text{ day}^{-1}) = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 1000$$

where W_1 and W_2 are the dry weights of the plants at times T_1 and T_2, respectively, and $T_2 - T_1$ is the interval of time in days between two observations.

For dry weight measurement the plants were harvested from 25 cm row lengths at two places in the second row on either side of each plot at 30, 60 and 90 days after sowing (DAS) and at the time of harvest. Air-dried samples were dried in an oven at 65±5°C till constant weight was achieved. Leaf water potential (ψ_L), osmotic potential (ψ_Π) and turgor potential (ψ_P) were measured concurrently on the flag leaves of plants at 95 DAS during the midday hours (12–14 h). The pressure chamber method, as described by Scholander et al. (1965), was used for recording leaf water potential. Two or three fully expanded flag leaves were excised from the base for measuring leaf water potential. After observation the same leaves were put inside airtight syringes and placed in a freezer at –15°C for leaf osmotic potential measurement. The sap from these leaf tissues was later expressed on a filter paper disc and the leaf osmotic potential was measured using a 5100-B vapour pressure osmometer. The leaf water potential and osmotic potential were used to determine the turgor potential from the equation:

$$\psi_P = \psi_L - \psi_\Pi$$

Various yield attributes were recorded at the time of harvest. The number of spikes per metre row length was counted at three randomly selected places in each plot. Ten representative spikes were harvested randomly from each plot for recording spike length, number of spikelets per spike, number of grains per spike and grain weight per spike. For recording 1000-grain weight, composite samples were taken from the produce of each plot. The grain and straw yields (kg ha^{-1}) were computed from a net undisturbed plot area of 5.0 × 4.2 m (gross plot area was 6.0 × 5.0 m).

Statistical analysis

Analysis of variance (ANOVA) at the 5% level of significance was used to evaluate the comparative performance of the factors (seed primings and varieties). To judge the significant difference between any two treatments, the critical difference (CD) was calculated according to Panse and Sukhatme (1995). Only the main effects of the treatments are given in the tables.

Results

Seedling emergence

The number of emerged seedlings was highest in the variety Sonak, followed by Raj 3765 and UP 2338, with the lowest value in PBW 343 at each stage of observation, i.e. 4, 8, 12 and 16 DAS in 1998–99 (Fig. 1a). The effect of the varieties on seedling emergence was not significant in 1999–2000 (Fig. 1b). The seedlings emerged earliest from sprouted seeds, followed by seeds soaked in IAA, KCl, water, $ZnSO_4$, and Na_2SO_4, in order and last in the control (Fig. 2a, 2b). About 90% of the total seedlings emerged by 8 DAS in the sprouted sowing and by 12 DAS in the other priming treatments, whereas the rate of emergence was very slow in the control in both the seasons. The number of seedlings which emerged was highest in the sprouted seeds and lowest in the non-primed seeds.

RGR

The variety Sonak exhibited significantly higher RGR between 0–30 and 30–60 DAS than the other varieties tested (Table 1), while at 60–90 DAS the varieties PBW 343, Raj 3765 and UP 2338 were at par with each other and had higher RGR than Sonak in 1998–99. However, during the next season the varietal effect on RGR was non-significant, except at 60–90 DAS. Among the different seed priming treatments, pre-germinated seed sowing showed significantly higher RGR, and lowest value was recorded in the control between 0–30 DAS; however, this trend was reversed between 60–90 DAS. The seed priming effect on RGR was non-significant during 30–60 DAS and 90 DAS–harvest.

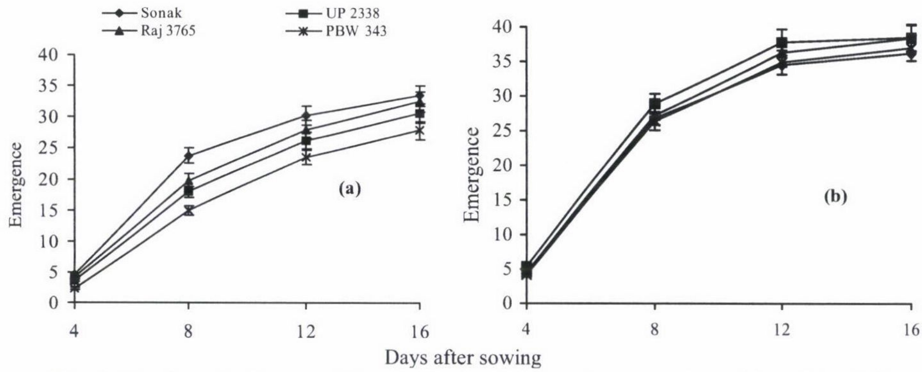

Fig. 1. Number of wheat seedlings emerging (per running metre) as affected by different varieties in 1998-99 (a) and 1999-2000 (b). The error bars represent the S.E. of the means.

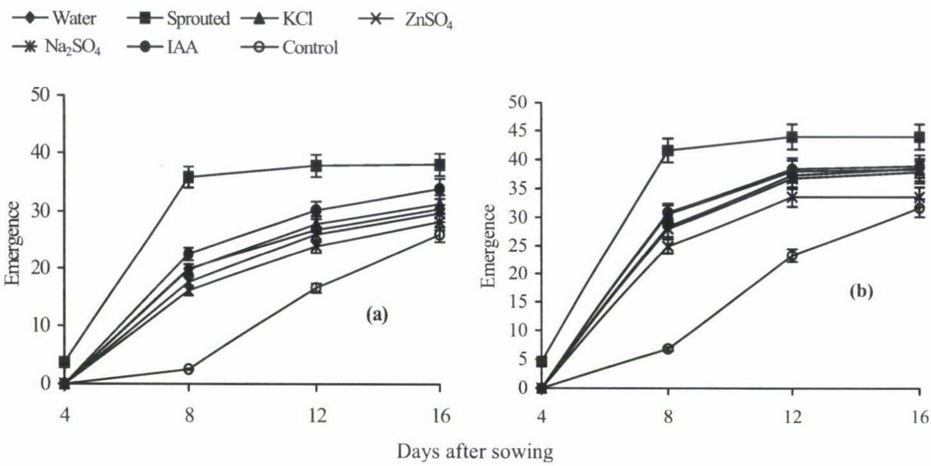

Fig. 2. Number of wheat seedlings emerging (per running metre) as affected by seed priming treatments in 1998–99 (a) and 1999–2000 (b). The error bars represent the S.E. of the means

Plant water relations

The leaf water potential, osmotic potential and turgor potential were highest in variety UP 2338, followed by Raj 3765 and PBW 343, and lowest in Sonak during both the growing seasons (Table 2). Priming with KCl resulted in maximum leaf water potential, followed by the IAA, $ZnSO_4$, water and Na_2SO_4 treatments and the control, with values significantly higher than those recorded when sowing sprouted seeds in both the years. The effect of the seed priming treatments on leaf osmotic potential was non-significant. The leaf turgor potential in priming treatments with KCl, $ZnSO_4$, Na_2SO_4 and IAA was higher than in the control or for sprouted seed.

Table 1

Effect of seed priming on relative growth rate (mg g^{-1} d^{-1}) of wheat varieties

Treatments	1998–99				1999–2000			
	Interval in days after sowing				Interval in days after sowing			
	0–30	30–60	60–90	90-h.	0–30	30–60	60–90	90-h.
Varieties								
Sonak	63.2	85.1	30.3	9.6	65.5	84.8	29.2	8.8
UP 2338	61.7	83.8	33.1	9.2	66.1	84.2	29.1	8.0
Raj 3765	62.2	82.7	33.5	9.9	66.6	82.8	30.5	8.1
PBW 343	60.8	80.3	36.1	9.6	65.3	84.1	30.4	8.5
SE m±	0.5	0.8	1.1	0.3	0.5	0.6	0.4	0.3
CD at 5%	1.5	2.4	3.2	NS	NS	NS	NS	NS
Seed primings								
Water	62.4	83.0	32.7	9.4	66.1	83.9	29.5	8.4
Sprouted	64.6	84.5	32.1	8.4	68.2	85.0	29.1	7.4
KCl	62.5	83.2	32.9	9.8	66.5	83.9	29.7	8.5
$ZnSO_4$	62.0	82.3	33.0	10.1	65.9	83.2	29.9	8.7
Na_2SO_4	61.5	82.5	33.2	10.1	65.5	83.3	30.0	8.8
IAA	63.2	83.2	32.8	9.1	66.9	83.9	29.8	8.0
Control	56.9	84.0	34.6	9.8	61.1	85.2	31.0	8.6
SE m±	1.1	0.5	0.6	0.4	1.0	0.4	0.5	0.3
CD at 5%	3.4	NS	1.5	NS	3.0	NS	1.4	NS

NS = Non-significant

Table 2

Effect of seed priming on leaf water potential (ψ_L), osmotic potential (ψ_Π) and turgor potential (ψ_P) of wheat varieties

Treatment	1998–99			1999–2000		
	ψ_L (–MPa)	ψ_Π (–MPa)	ψ_P (+MPa)	ψ_L (–MPa)	ψ_Π (–MPa)	ψ_P (+MPa)
Varieties						
Sonak	1.91	2.37	0.46	1.74	2.39	0.55
UP 2338	1.51	2.15	0.64	1.51	2.20	0.67
Raj 3765	1.60	2.20	0.60	1.55	2.24	0.69
PBW 343	1.82	2.30	0.48	1.70	2.32	0.62
SE m±	0.02	0.20	0.01	0.01	0.01	0.01
CD at 5%	0.06	0.06	0.04	0.05	0.04	0.03
Seed primings						
Water	1.71	2.24	0.53	1.70	2.25	0.55
Sprouted	1.84	2.27	0.43	1.77	2.28	0.51
KCl	1.60	2.22	0.62	1.57	2.26	0.69
$ZnSO_4$	1.70	2.31	0.61	1.67	2.33	0.66
Na_2SO_4	1.73	2.31	0.58	1.71	2.32	0.61
IAA	1.65	2.22	0.57	1.63	2.23	0.60
Control	1.73	2.21	0.47	1.71	2.24	0.53
SE m±	0.01	0.01	0.02	0.01	0.01	0.01
CD at 5%	0.04	NS	0.05	0.04	NS	0.04

NS = Non-significant

Yield attributes

The number of spikes per running metre was highest in variety PBW 343 and lowest in Sonak in 1999–2000 (Table 3). The variety UP 2338 produced significantly longer spikes than the other varieties in 1998–99, while the spikes of Sonak were the longest in the succeeding year. The number of spikelets per spike and number of grains per spike were maximum in variety UP 2338 and minimum in Sonak. The varieties did not differ significantly for grain weight per spike in the first year, whereas in the successive year it was lowest in Sonak. The test weight (1000-grain weight) of varieties Raj 3765 and Sonak was highest, while that of UP 2338 was lowest. All the characters attributing to yield were highest in the sprouted seeds, followed by those in the various priming treatments (IAA, KCl, water, $ZnSO_4$, Na_2SO_4), compared with the control.

Table 3
Effect of seed priming on yield attributes of wheat varieties

Treatment	1	2	3	4	5	6
Varieties			1998–99			
Sonak	73.9	9.70	14.10	37.69	1.62	43.43
UP 2338	76.0	10.00	19.24	50.98	1.88	39.58
Raj 3765	77.6	9.48	15.88	41.47	1.86	43.60
PBW 343	70.4	9.41	17.88	41.84	1.77	41.60
SE m±	1.1	0.09	0.17	0.57	0.03	0.34
CD at 5%	NS	0.31	0.60	1.96	NS	1.17
Seed primings						
Water	73.5	9.71	16.96	43.17	1.78	42.00
Sprouted	84.5	10.16	17.80	46.23	1.93	42.90
KCl	76.6	9.83	17.11	42.83	1.80	42.16
$ZnSO_4$	72.8	9.60	16.58	42.47	1.76	41.60
Na_2SO_4	70.2	9.45	16.43	42.43	1.74	41.40
IAA	78.4	9.89	17.12	44.44	1.83	42.50
Control	65.4	8.85	15.40	38.41	1.60	41.70
SE m±	0.8	0.09	0.14	0.45	0.02	0.20
CD at 5%	2.4	0.28	0.50	1.26	0.05	0.56
Varieties			1999–2000			
Sonak	83.1	11.00	15.05	43.62	1.58	42.98
UP 2338	92.1	10.12	19.74	54.70	1.82	38.65
Raj 3765	92.6	10.24	17.70	45.78	1.81	43.20
PBW 343	95.0	10.10	19.00	50.43	1.80	41.74
SE m±	0.7	0.10	0.12	0.30	0.02	0.23
CD at 5%	2.4	0.34	0.42	1.05	0.07	0.80
Seed primings						
Water	89.3	10.42	18.14	48.88	.76	41.62
Sprouted	99.4	10.77	18.57	51.90	1.89	42.40
KCl	93.9	10.51	18.00	49.67	1.80	41.85
$ZnSO_4$	89.0	10.37	17.67	48.18	1.72	41.52
Na_2SO_4	87.6	10.21	17.54	47.18	1.69	41.07
IAA	95.0	10.55	18.30	50.64	1.81	41.96
Control	80.8	9.70	17.00	44.10	1.59	40.98
SE m±	1.0	0.14	0.14	0.24	0.02	0.20
CD at 5%	2.9	0.39	0.40	0.70	0.06	0.57

1: No. of spikes(m^{-1}rl), 2: Spike length (cm), 3: No. of spikelets/spike, 4: No. of grains/spike, 5: Grain weight/spike (g): 6: 1000-grain weight (g)

Yield

The grain and straw yields were better in the 1999–2000 crop season than in 1998–99 (Table 4). In the year 1998–99, the grain yield did not exhibit varietal differences, while the straw yield of UP 2338 was highest and that of PBW 343 lowest. In the successive season the maximum grain and straw yields were obtained in PBW 343 and the minimum in Sonak. Like the various yield attributes, the grain and straw yields were highest in the sprouted seeds; the next best was priming with IAA, KCl and water, followed by $ZnSO_4$ and Na_2SO_4; the lowest yield was obtained in non-primed seeds.

Discussion

In primed seeds, the early emergence of seedlings was due to the prior initiation of the imbibition process during soaking (Kahlon et al., 1992). The relative growth rate was higher in primed treatments than for dry seed between 0–30 DAS due to rapid seedling establishment, better crop stand, vigorous plants and fast growth (Harris et al., 2001; Musa et al., 2001). The effect of seed priming on RGR was reversed between 60–90 DAS, because vegetative growth was completed earlier in primed treatments, while in unprimed seeds the dry matter accumulation persisted longer due to initial slow vegetative growth. The improved values of leaf water potential and turgor potential after seed priming with salts might be due to the greater hydration of the colloids and lower water deficits (Das, 1997), thus maintaining plant water status.

Table 4

Effect of seed priming on grain and straw yield (kg ha^{-1}) of wheat varieties

Treatment	1998–99		1999–2000	
	Grain yield	Straw yield	Grain yield	Straw yield
Varieties				
Sonak	4250	6141	4280	6486
UP 2338	4296	6390	4420	6718
Raj 3765	4443	6320	4464	6775
PBW 343	4162	5819	4547	6862
SE m$\pm$	20	38	24	39
CD at 5%	NS	131	81	134
Seed primings				
Water	4307	6192	4401	6615
Sprouted	4697	6659	4796	7236
KCl	4365	6252	4470	6747
$ZnSO_4$	4187	6132	4389	6593
Na_2SO_4	4123	5974	4330	6530
IAA	4476	6441	4559	6836
Control	3858	5523	4048	6416
SE m$\pm$	65	88	47	67
CD at 5%	187	250	135	188

NS = Non-significant

The priming treatments were found to be beneficial in improving the yield attributes and obtaining higher grain and straw yields as compared to the control (Tables 3 and 4). The higher yields recorded with seed priming were due to quick emergence, better seedling establishment and optimum growth rate, as observed earlier by Bhati and Rathore (1986) and Musa et al. (2001). The additive effects of IAA priming over water soaking may be due to the fact that low temperature suppressed the activity of naturally present growth hormones, while priming fulfilled the optimum requirement exogenously (Chhipa and Lal, 1978), as IAA possibly helps in better root growth and increased water absorption (Darra et al., 1973). Priming with KCl was beneficial, since potassium plays an important role in physiological processes such as osmoregulation and turgor maintenance (Zaidi et al., 1994).

Seedling emergence was highest in the variety Sonak and lowest in PBW 343 in 1998–99 (Fig. 1a), because of their genetic characteristics and differential responses to diverse environmental conditions, whereas in 1999–2000 the effect of the varieties on seedling emergence was non-significant (Fig. 1b), due to the comparatively favourable day temperatures and sunshine hours for three to four weeks after sowing (data not shown). These favourable environmental conditions improved emergence primarily in varieties PBW 343 and UP 2338. In 1998–99 the RGR was highest in variety Sonak at 0–30 and 30–60 DAS, because of its short duration and early establishment under late-sown conditions.

The effect of the varieties on grain yield was non-significant in 1998–99, but the straw yield of varieties UP 2338 and Raj 3765 was higher than that of Sonak and PBW 343 (Table 4). The varieties UP 2338 and Raj 3765 produced higher leaf area and total biomass accumulation (data not shown) in the first year. The higher productivity of PBW 343 in 1999–2000 compared to the other varieties was due to the larger number of effective tillers (spikes) at harvest (Table 3). The variety PBW 343 showed a remarkable difference in grain and straw yields during the two growing seasons. The reasons for its low productivity in 1998–99 were poor germination, delayed emergence and the suppression of vegetative growth. Significant variations in emergence, growth and yield were apparent between the different varieties under late-sown conditions due to genetic divergence (Nainwal and Singh, 2000; Singh and Uttam, 1994).

The beneficial results of seed soaking in different salts and the sowing of sprouted seeds were quite evident in the field, especially under the adverse climate of delayed sowing. These results are of direct relevance when sowing is done manually, while some problems may arise when mechanical seed drills are used. The soaked seeds swell up and will change the planting rates and efficiency of delivery. In the case of soaked seeds, changing the size of the controlling aperture may solve the problem, whereas pre-germinated seeds are susceptible to physical damage. Hence, priming in aqueous solution is the next best idea. Since water soaking was as good as the other chemical treatments and is cheap, convenient and simple, it seems logical for farmers to prime seed with water. Further research will be necessary, however, to estimate the rate of seed injury when sprouted seeds are sown with mechanical seed drills. The problem may be overcome by increasing the seed rate or by developing suitable mechanical planters.

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Short communication

HPLC ANALYSIS OF CAROTENOIDS FROM FRUITS OF *CUCURBITA PEPO* L. VAR. *MELOPEPO* ALEF.

E. MUNTEAN, C. BELE and C. SOCACIU

UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE, CLUJ NAPOCA, ROMANIA

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The fruits of *Cucurbita pepo* L. var. *melopepo* Alef. are very much appreciated in low calory human diets, mainly for their delicate taste. Their carotenoid pattern has not yet been established, as both the epicarp and the mesocarp are white, suggesting that they contain no carotenoids. HPLC analysis of carotenoids from these fruits revealed a chromatographic pattern very similar to that of other fruits belonging to different varieties of *Cucurbita pepo* L. - a fact with great chemotaxonomic importance. The main carotenoids are lutein and β-carotene; traces of violaxanthin, lactucaxanthin, β-cryptoxanthin, 9Z-β-carotene and 15Z-β-carotene are also present. HPLC separation was achieved on a Nucleosil 120 - 5 C_{18} column, using the following mobile phases: A – acetonitrile : water (9 : 1) and B – ethyl acetate. The flow rate was 1 ml/min and the solvent gradient was as follows: from 0 to 16 min - 10 to 70% B, then from 16 to 25 min – 70 to 10% B. Quantification of the carotenoids was achieved by the internal standard method, using echinenone as internal standard. The total carotenoid content was found to be 1.12 μg carotenoids/g dry weight for the mesocarp and 3.62 μg carotenoids/g dry weight for the epicarp.

Key words: HPLC, chromatography, carotenoids, scallop, crown gourd, *Cucurbita pepo* L.

Introduction

The need for reliable data on the carotenoid content of food products has become increasingly urgent since a possible link has been detected between carotenoid intake and health. Plants belonging to the genus *Cucurbita*, besides having considerable dietary and feeding value, host notable amounts of carotenoids in their fruits (Arima et al., 1988; Hidaka et al., 1987; Khachik et al., 1988; Lee et al., 1984; Muntean and Rotar, 2001; Neamtu et al., 1985). *Cucurbita pepo* L. var. *melopepo* Alef. fruits (crown gourd or scallop squash) are not dietary sources of carotenoids, as indicated by the colour of their epicarp and mesocarp, which are both white. Despite appearances, the carotenoid content of these fruits was checked, working on a large-scale extraction, and traces of carotenoids became visible. By means of HPLC analysis, their chromatographic profile was obtained.

Materials and methods

Reagents and materials: Carotenoid standards were kindly provided by F. Hoffman (La Roche, Basel, Switzerland). All solvents contained 0.1% butylated hydroxytoluene. The solvents used in HPLC analysis were of HPLC grade (ROMIL Chemicals); they were filtered through 0.5 μm microfiber filters (Whatman), then degassed using an ultrasonic bath, under vacuum, before use. Solvents for extraction were freshly distilled.

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Special precautions: All operations were carried out in reduced light, avoiding sample heating to more than 40°C; prior to injection in HPLC systems, the carotenoid solutions were filtered through 0.45 μm Whatman filters.

Plant material: Immature fruits were harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca in July, 2002; the epicarp was peeled, then the epicarp and mesocarp were cut into small pieces, packed in sealed polyethylene bags, weighed, and stored at –25°C until analysis.

Extraction and saponification: Carotenoids from the epicarp and mesocarp (90–100 g samples) were extracted in a blender using 300 ml methanol, to which 1 g butylated hydroxytoluene (BHT) and 10 g $CaCO_3$ were added to avoid oxidation and acidic isomerisation during the extraction procedure. At this stage, an appropriate amount of echinenone solution (internal standard) was added to each sample. The resulting mixture was filtered under suction with a sintered-glass funnel and the solid material was re-extracted twice with 100 ml acetone. The resulting extract was washed ten times with distilled water, concentrated under reduced pressure in a Buchi rotary evaporator (at 40°C) and dissolved in 25 ml diethyl ether. A 5 ml aliquot was used for HPLC and the remaining extract was saponified with 25 ml 30% KOH solution in methanol at room temperature for 16 hours (overnight). The unsaponifiable fraction was next extracted with petroleum ether and washed repeatedly with distilled water until free of alkali; the aqueous layers were re-extracted with 50 ml diethyl ether, then the organic layers were combined, washed several times with distilled water and evaporated to dryness under reduced pressure. The saponified extract was dissolved in 5 ml ethyl acetate and an aliquot was used for HPLC. The extraction procedure was in conformity with the literature requirements (Britton et al., 1995).

HPLC separations were performed on a system consisting of a Kontron Instruments pumping system 322, a Rheodyne 7152 injection valve with a 20 μl loop and a Waters 990 photodiode array detector connected to a 80386 computer using WATERS 990 software for data analysis. Separations were carried out on a Nucleosil 120 - $5C_{18}$ column (250 × 4.6 mm, 5 μm particle size), protected with a guard column containing C_{18} material similar to the analytical column. The carotenoids were separated at room temperature, under gradient conditions, using 10% A, 90% B from 0–16 min, followed by min. 90% A, 10% B from 16 to 25 min. A being a mixture of acetonitrile : water (9:1) and B being ethyl acetate (both A and B contained 0.5% EPA). The separations were monitored at 450 nm. Quantification of the carotenoids was achieved by the internal standard method, using echinenone as internal standard.

Identification of carotenoids was made on the basis of diode array spectral characteristics, retention times, co-chromatography with authentic standards, relative elution order compared to authentic standards and data from the literature (Britton et al., 1996; Davies, 1976).

Results and discussion

The total carotenoid content of the analysed fruits was found to be 0.122 μg carotenoids/g fresh weight (1.117 μg carotenoids/g dry weight) for the mesocarp, and 0.46 μg carotenoids/g fresh weight (3.62 μg carotenoids/g dry weight) for the epicarp.

HPLC separations were difficult because of the high amount of lipids extracted; as a result, β,β-carotene and its isomers were not separated and were quantified together.

The chromatographic profiles revealed that the main carotenoids were lutein and β,β-carotene for both the epicarp and the mesocarp (Figs. 1 and 3). Small amounts of violaxanthin, lactucaxanthin, β-cryptoxanthin, 9Z-β,β-carotene and 15Z-β,β-carotene were also present. The chromatograms of the unsaponified total extract showed the presence of chlorophylls b, b', a, a' and of pheophytin a and b (Figs. 2 and 4); the identities of these compounds and their distribution between the fruit mesocarp and epicarp are presented in Table 1.

Table 1
Distribution of carotenoids in fruits of *Cucurbita pepo* L var. *melopepo* Alef.

Peak index	Compound	In mesocarp (µg/g dry weight)	In epicarp (µg/g dry weight)
1	Violaxanthin	traces	traces
2	Lactucaxanthin	traces	traces
3	Lutein	0.45	2.74
4	β-cryptoxanthin	traces	traces
5	β,β-carotene	0.63	0.76
6	9Z-β,β-carotene	0.63	0.76
7	15Z-β,β-carotene	0.63	0.76
C1	Chlorophyll a	present	present
C2	Chlorophyll a'	present	present
C3	Chlorophyll b	present	present
C4	Chlorophyll b'	present	present
F1	Pheophytin a	present	present
F2	Pheophytin b	–	present

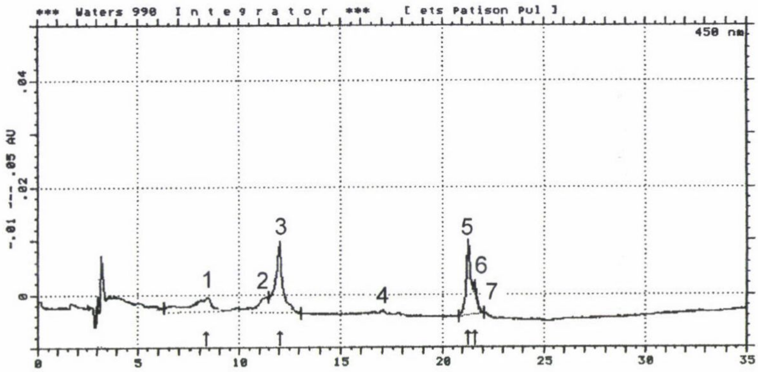

Fig. 1. HPLC chromatogram of the total saponified extract from the mesocarp of *Cucurbita pepo* L. var. *melopepo* Alef. fruits (peak identities in Table 1)

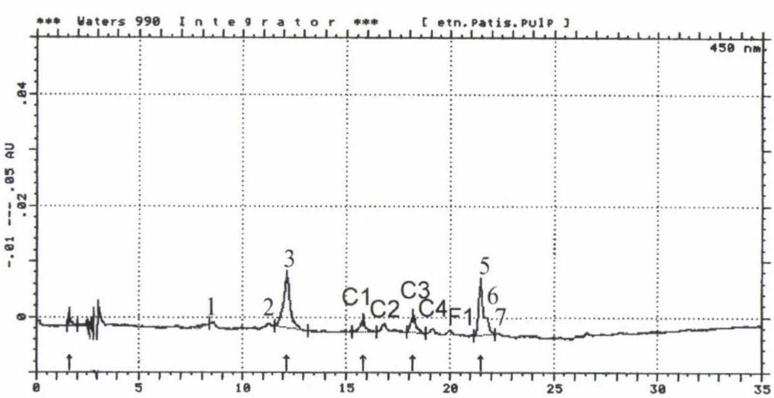

Fig. 2. HPLC chromatogram of the unsaponified total extract from the mesocarp of *Cucurbita pepo* L. var. *melopepo* Alef. fruits (peak identities in Table 1)

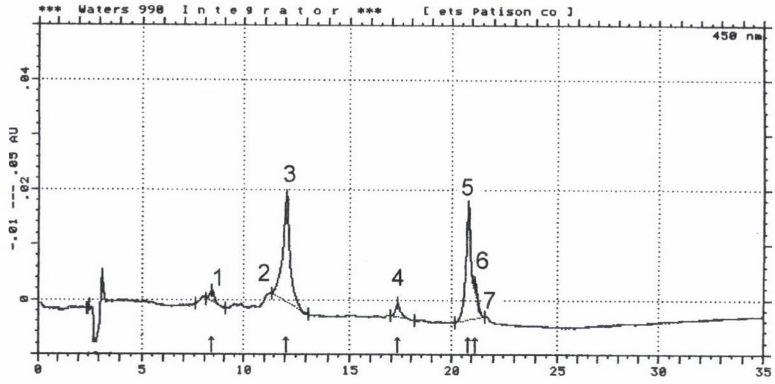

Fig. 3. HPLC chromatogram of the total saponified extract from the epicarp of *Cucurbita pepo* L. var. *melopepo Alef.* fruits (peak identities in Table 1)

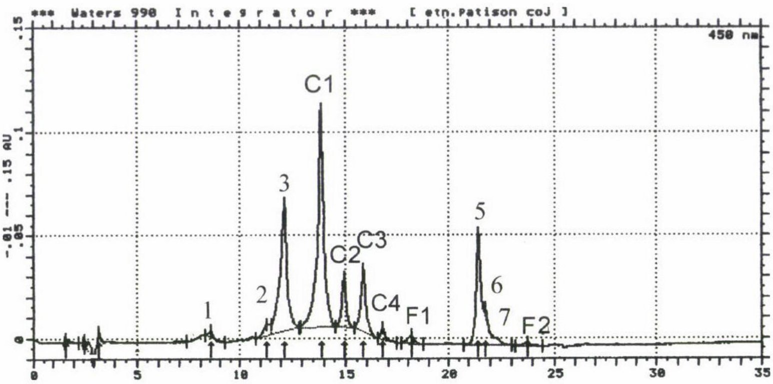

Fig. 4. HPLC chromatogram of the unsaponified total extract from the epicarp of *Cucurbita pepo* L. var. *melopepo* Alef. fruits (peak identities in Table 1)

Conclusions

This study revealed the chromatographic profile of the carotenoids from fruits of *Cucurbita pepo* L. var. *melopepo* Alef., being the first investigation on this variety. HPLC analysis revealed that, despite these fruits being white, they contain detectable amounts of carotenoids; the quantitative data indicated a higher concentration of carotenoids in the epicarp than in the mesocarp (Table 1).

HPLC analysis revealed that the major carotenoids were β,β-carotene and lutein, besides which traces of lactucaxanthin, β-cryptoxanthin, 9Z-β-carotene and 15Z-β-carotene were also detected. HPLC also revealed that all the xanthophylls were present in a free, non-esterified form.

Comparison of the carotenoid HPLC chromatographic profiles of fruit of this variety with those belonging to other varieties of *Cucurbita pepo* L. (Muntean, 2003) revealed a similar chromatographic pattern, a fact with great chemotaxonomic importance.

Acknowledgements

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Short communication

EFFECT OF WEED MANAGEMENT PRACTICES ON THE YIELD ATTRIBUTES AND YIELD OF WET-SEEDED RICE

M. RAJU, B. J. PANDIAN, P. THUKKAIYANNAN and N. THAVAPRAKASH

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Field experiments were conducted at the Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, India during the *kharif* (July to November) and summer (December to April) seasons of 1999 and 2000 in a randomized block design. The treatment consisted of three pre-emergence herbicides (pretilachlor + safener 0.3 kg ha^{-1} 4 days after sowing [DAS], butachlor 1.0 kg ha^{-1} 8 DAS and pendimethalin 1.0 kg ha^{-1} 8 DAS) and one early post-emergence herbicide (butanil 3.0 ha^{-1} 15 DAS), each in combination with mechanical or hand weeding 30 and 45 DAS. In addition, green manure (Daincha) intercropping and incorporation, mechanical and hand weeding twice alone (25 and 50 DAS) were compared with the unweeded check. The results revealed that the pre-emergence application of pretilachlor + safener 0.3 kg ha^{-1} + hand weeding twice (30 and 45 DAS) promoted higher yield attributes and maximum yield in wet-seeded rice.

Key words: wet-seeded rice, weed management, yield attributes, yield

Introduction

Rice occupies a pivotal position in the food security system of Asian countries. The future of the food security system will depend on our ability to achieve a continuous improvement in the productivity of rice. Depending on the season and the variety, rice is grown under varying environments, including terrestrial, semi-aquatic and aquatic conditions. Transplanting rice is the traditional system of rice crop establishment and is still in vogue in many rice-growing areas. However, with the availability of short duration varieties and effective herbicides, and due to the non-availability and escalating costs of labour, the adoption of wet-seeded rice could be a viable alternative to transplanted rice. Weed management is one of the most critical factors, since wet seeding favours the simultaneous germination of weed seeds along with paddy seeds (Martin, 1998). Weeds are the only universal pest in rice and cause a yield loss of 72.5% in direct seeded rice (Kolhe and Tripathi, 1998). Unless adequate weed management practice is applied, higher yields cannot be achieved. Jayadeva and Nanjappa (1996) reported that the highest grain yield of 5.02 t ha^{-1} was obtained with the application of 1.0 kg ha^{-1} pretilachlor + safener 3 DAS in direct-seeded puddled rice. The highest grain yield of 5.94 t ha^{-1} was recorded with butachlor at 1.5 kg ha^{-1} applied one day before sowing in direct-sown rice

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on puddled soils (Natarajan and Kuppuswamy, 1997). The application of pendimethalin at 1.0 kg ha^{-1} recorded the highest grain yield in upland direct seeded rice (Pandey and Tiwari, 1996). The control of weeds in wet-seeded rice through the proper adoption of weed management practices is considered crucial for maximizing the grain yield. The present study was thus undertaken to study the effect of weed management practices on the yield attributes and yield of wet-seeded rice.

Materials and methods

Field experiments on wet-seeded rice were carried out at the Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, India during the *kharif* (July to November) and summer (December to April) seasons of 1999 and 2000. Killukulam, Tamil Nadu is situated at 8°N latitude and 77° E longitude at an altitude of 40 m above mean sea level.

The soil of the experimental field was fine, well-drained, sandy clay loam in texture with low available nitrogen (176 kg ha^{-1}), high available phosphorus (27 kg ha^{-1}) and medium available potassium (126 kg ha^{-1}). The electrical conductivity of the soil was 0.21 dS m^{-1} and the pH was 6.8. Mechanical analysis of the soil showed 16.7, 25.3, 26.5 and 30.2% of silt, clay, fine sand and coarse sand, respectively. The rice variety ADT 36, with a field duration of 105 days, was used in the trial.

The experiment was laid out in a randomized block design replicated three times. Treatment details are furnished below:

T_1 Pretilachlor + safener 0.3 kg ha^{-1} applied 4 DAS followed by (fb) mechanical weeding 30 and 45 DAS
T_2 Pretilachlor + safener 0.3 kg ha^{-1} 4 DAS fb hand weeding 30 and 45 DAS
T_3 Butachlor 1.0 kg ha^{-1} 8 DAS fb mechanical weeding 30 and 45 DAS
T_4 Butachlor 1.0 kg ha^{-1} 8 DAS fb hand weeding 30 and 45 DAS
T_5 Pendimethalin 1.0 kg ha^{-1} 8 DAS fb mechanical weeding 30 and 45 DAS
T_6 Pendimethalin 1.0 kg ha^{-1} 8 DAS fb hand weeding 30 and 45 DAS
T_7 Butanil 3.0 lit ha^{-1} 15 DAS fb mechanical weeding 30 and 45 DAS
T_8 Butanil 3.0 lit ha^{-1} 15 DAS fb hand weeding 30 and 45 DAS
T_9 Green manure, Dhaincha (*Sesbania aculeata*) as intercrop and *in situ* incorporation
T_{10} Mechanical weeding 25 and 50 DAS
T_{11} Hand weeding 25 and 50 DAS
T_{12} Unweeded check (control)

A fertilizer schedule of 100 kg N, 50 kg P and 50 kg K ha^{-1} was applied to all plots. The entire phosphorus and 50% of the nitrogen and potassium fertilizers were applied basally. The remaining nitrogen was applied in three splits, 10 DAS, and at the tillering and panicle initiation stages. The remaining 50% K was applied in two splits at the tillering and panicle initiation stages. Urea, superphosphate and muriate of potash were used for supplying N, P and K, respectively.

The herbicide treatments were scheduled at the appropriate time as mentioned in the treatment schedule. Dhaincha (*Sesbania aculeata*) was raised as an intercrop because it produces more foliage and was incorporated mechanically 40 DAS.

The panicle-bearing tillers were counted at random by placing four quadrates in each plot to calculate the productive tillers m^{-2}. The number of well-filled grains from the labelled panicles were counted and averaged out to get the number of filled grains $panicle^{-1}$. A sample of a thousand grains was taken from each net plot and grain yield was recorded at 12% moisture content and expressed as kg ha^{-1}. Statistical analysis was carried out for the two seasons of the trial and the data are presented in Table 1.

Table 1
Effect of weed management practices on the yield and yield attributes of wet-seeded rice

Treatments	Plant population (No. m^{-2})		Productive tillers (m^{-2})		No. of filled grains panicle^{-1}		1000-grain weight (g)		Straw yield (t ha^{-1})		Grain yield (t ha^{-1})	
	K	S	K	S	K	S	K	S	K	S	K	S
T_1	291.6	277.0	343.0	351.6	80.0	81.7	22.4	22.3	5.8	5.3	4.6	4.3
T_2	306.3	271.6	409.0	374.0	85.3	87.6	22.9	23.0	7.2	6.3	5.7	5.1
T_3	270.0	252.0	319.3	307.3	74.8	73.4	22.7	22.2	5.4	4.8	4.4	3.9
T_4	266.6	256.0	323.3	312.6	77.0	75.9	22.7	22.5	5.6	5.3	4.5	4.3
T_5	271.3	269.6	336.0	319.6	81.6	79.8	22.7	22.6	6.1	5.5	5.0	4.5
T_6	276.3	272.3	342.0	323.3	82.1	80.1	22.8	22.6	6.7	5.9	5.5	4.8
T_7	271.3	264.3	334.0	327.3	78.2	70.4	22.3	22.3	5.7	5.2	4.7	4.2
T_8	274.0	266.3	338.0	335.6	79.6	71.9	22.4	22.4	5.8	5.1	4.8	4.1
T_9	261.3	251.3	306.0	305.0	72.0	67.2	22.2	22.2	5.2	4.7	4.2	3.8
T_{10}	268.0	260.0	317.0	301.6	74.0	73.0	22.2	22.2	5.5	4.9	4.5	4.0
T_{11}	273.6	266.3	323.0	334.6	78.4	76.2	22.4	22.4	6.2	5.6	5.0	4.6
T_{12}	252.3	249.6	264.3	262.67	61.0	62.6	21.4	21.6	3.2	2.8	2.4	2.0
$LSD_{P=0.05}$	9.08	8.16	10.67	9.12	1.02	0.85	0.45	0.49	3.4	2.0	2.2	1.5

K: Kharif, S: Summer

Results and discussion

Yield attributes of wet-seeded rice

The yield attributes, productive tillers m^{-2}, number of filled grains panicle^{-1} and 1000 grain weight, were significantly higher in the treatment involving the pre-emergence application of pretilachlor followed by hand weeding twice. This appears to have been the result of effective, prolonged weed control. Similar results were also reported by AICRPWC (1993).

The phytotoxic effect exerted by butachlor at the early growth stage of the crop was evident from the lower plant stand and reduced growth characters, which in turn resulted in a reduction in productive tillers, as also reported by Angiras and Rana (1998).

All the weed management practices significantly enhanced the number of filled grains panicle^{-1}. The greatest number of filled grains panicle^{-1} was recorded with pretilachlor followed by hand weeding twice. Similar results were also reported by Kondap et al. (1985).

Yield of wet-seeded rice

Wet-seeded rice yielded more in the *kharif* than in the summer season. The higher yield obtained in the *kharif* season was mainly due to the lower weed population as compared to summer. The wet-seeded rice yield was significantly influenced by different weed management practices. The pre-emergence application of pretilachlor + safener at 0.3 kg ha^{-1} followed by hand weeding 30 and 45 DAS produced a significantly higher grain yield of 5733 and 5150 kg ha^{-1} in the *kharif* and summer seasons, respectively. The straw yield was also promoted, showing a similar trend in both seasons. Similar findings were reported by Ramamoorthy and Balasubramanian (1995).

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